





LABORATORY OF INFECTIOUS DISEASES
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Summary Statement
LABORATORY OF INFECTIOUS DISEASES
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RESPIRATORY VIRUSES SECTION

Development of Live Attenuated RSV Vaccines. Respiratory syncytial virus (RSV) is the most common cause of severe viral lower respiratory tract disease in infants and children world wide, and, for this reason, there is a need to develop a vaccine that prevents serious illness requiring hospitalization. In the 1960s, a formalin-inactivated RSV (FI-RSV) vaccine was found to be unsatisfactory because it failed to protect against RSV infection, and unexpectedly, it potentiated disease at a later time when vaccinees were naturally infected with RSV. As a consequence, during the 1970's - early 80's, our laboratory redirected its efforts at prophylaxis to the development of live attenuated RSV vaccine strains. These efforts were temporarily abandoned because of a failure to produce vaccine strains that were stable genetically and satisfactorily attenuated for seronegative infants and young children. Subsequently, we systematically evaluated RSV subunit protein vaccines, vaccinia-RSV recombinant viruses, and adenovirus-RSV recombinant viruses in rodents and nonhuman primates. These studies led us to conclude that within the constraints of current technology these approaches are not likely to yield a successful RSV vaccine. Therefore, we returned to our original objective which was the development of a live attenuated RSV vaccine. It is now clear that RSV strains exist as two antigenically related subgroups, subgroup A and B, that are 25% related by cross-neutralization. Hence, it is likely that a bivalent formulation containing both subgroup A and subgroup B vaccine strains will be required to provide broad resistance.

Recently, significant progress has been made in the development of a live attenuated RSV subgroup A vaccine by using chemical mutagenesis to introduce additional attenuating mutations into mutants, such as cold passage (*cp*) RSV (*cp*RSV), that were not completely attenuated for fully susceptible infants and young children. In this manner several promising candidate RSV subgroup A vaccine candidates were produced and then evaluated in experimental animals. The cold-passage temperature-sensitive (*cpts*) 248/955, 248/404, and 530/1009 mutants derived from *cp*RSV were selected from a large panel of mutants for further study because they were attenuated, stable genetically, immunogenic, and able to induce protective immunity in rodents or chimpanzees. Importantly, these mutants were able to induce a high level of resistance to wild type virus challenge even in chimpanzees passively infused with RSV antibodies at the time of immunization, a situation which simulated that of the young human infant whose serum usually contains a moderate level of passively acquired maternal RSV antibodies. The nucleotide sequences of the most promising attenuated viruses are being determined while that of the *cp*RSV parent mutant was completed this year.

The *cpts*248/955 mutant was found to be very stable genetically in seronegative infants and young children, but it was not completely attenuated in these vaccinees. Importantly, the *cpts*530/1009 mutant appeared to be both satisfactorily attenuated and stable genetically in the seven infected vaccinees evaluated thus far. Thus, the *cpts*530/1009 vaccine is our most promising candidate to date for the subgroup A component of a bivalent RSV vaccine.

Progress toward the development of the subgroup B component of the bivalent RSV vaccine was also made this year. A subgroup B RSV (strain B1) was passaged 52 times at low temperature over a period of two years. The resulting mutant, cold passage 52 RSV B1, was restricted in its replication in the respiratory tract of cotton rats. Evaluation of mutants selected at different passage levels suggested that the *cp52* mutant sustained three independent mutations that singly or in combination contributed to its attenuation for cotton rats. The attenuation phenotype of this mutant was highly stable even after prolonged replication in immunosuppressed cotton rats. Derivatives of the *cp52*RSV B1 mutant were obtained following two successive rounds of chemical mutagenesis and two *ts* mutants were selected from a large number of candidates for further study, namely *cp*RSV B1 176 and *cp*RSV B1 176/427. These mutants are now being evaluated in clinical trials because they exhibit a level of temperature sensitivity known to be associated with attenuation of other respiratory tract viruses *in vivo*.

Rescue of Infectious RSV from Full-Length RSV cDNA. Previously an experimental "rescue" system was developed for RSV based on a cDNA-encoded "minigenome", called RSV-CAT RNA, bearing the foreign marker gene for chloramphenicol acetyl transferase (CAT) flanked by 3' and 5' terminal *cis*-acting RSV non-coding sequences. When synthesized *in vitro* and transfected into RSV-infected cells, RSV-CAT RNA was competent for RSV-specific transcription, replication and incorporation into virions. This represents the first type of system in which genome-like RNAs of a nonsegmented negative strand RNA virus could be introduced into the viral replication cycle and rendered biologically active. This system was used successfully to identify and characterize the *cis*-acting replication and transcription signals in the RSV genome and to study the mechanism of gene expression.

Recently, this system was modified successfully such that RSV infection as the source of complementing proteins was replaced by RSV proteins expressed from transfected cDNAs. Thus, the mix and relative amounts of complementing proteins could be varied. Three proteins, N, P and L, which make up the nucleocapsid complex, were necessary and sufficient for RNA replication, *i.e.*, the synthesis of genome and antigenome [positive-sense replicative intermediate] RNAs. However, transcription (synthesis of subgenomic mRNA) by these three proteins alone yielded prematurely terminated mRNA. The coexpression of catalytic amounts of the M2 mRNA restored authentic transcription. Thus, the RSV "replicase" and "transcriptase" are not identical; the latter requires an additional elongation factor (viral M2 protein). At high concentrations of input M2 cDNA (yielding levels of protein comparable to those observed late during RSV infection), both transcription and RNA replication were inhibited. The M2 mRNA contains two overlapping open reading frames (orfs): the upstream orf encodes the viral membrane-associated M2 protein, and the second, internal orf lacks a known protein product. The effect on transcriptional elongation mapped to the former; the effect of inhibition of RNA synthesis to the latter. Coexpression (with N, P and L) of the NS1 protein resulted in inhibition of the synthesis of all RNAs, whereas coexpression of NS2 inhibited the synthesis of positive-sense RNAs. The M, SH, G and F proteins did not produce a detectable effect on RNA synthesis. Preliminary experiments indicated that transmissible particles were made when the mix of complementing proteins included the four envelope-associated proteins, M, SH, F and G, in addition to N, P, L and M2.

At this time, attenuated strains of RSV, such as those described above, appear to hold the most promise for vaccine development. One problem is that the development of these strains by cold-passages or chemical mutagenesis is a hit-or-miss process. Among mutations identified by sequence analysis, those responsible for desirable phenotypes cannot be distinguished from

incidental mutations acquired during passage. Importantly, mutations conferring desirable phenotypes cannot be combined. Also, even if an ideal vaccine virus can be produced, it might be rendered less effective as a consequence of antigenic drift among circulating viruses. Thus, the characterization and production of attenuated strains would benefit greatly from development of a method for producing RSV from cloned cDNA.

Full length negative strand RSV RNA is not infectious by itself. This means that complementing viral proteins are required within the host cell to transcribe and replicate full length viral RNA in order to initiate infection. This was achieved for the first time during the past year and has provided us with the first available method for producing live infectious RSV from cloned cDNA. Infectious RSV was produced by the intracellular coexpression of five cDNAs encoding: (i) a complete version of RSV replicative intermediate RNA, and (ii) the four RSV proteins identified and cited above as being necessary and sufficient to produce nucleocapsids competent for transcription and RNA replication. This represents a watershed in the molecular studies of RSV initiated in the LID fifteen years ago and provides us with the first available method for the direct engineering of infectious RSV.

One important use of this new technology will be to facilitate and extend the characterization and development of a live attenuated RSV vaccine based on existing candidate vaccine strains. Specifically, the mutations responsible for desired phenotypic characteristics (attenuation, temperature-sensitivity, cold-adaptation, small plaque size, host range restriction, etc.) of these existing viruses can now be identified directly by inserting a single mutation or a combination of mutations into the "wild type" genome and characterizing the resulting mutants. Desirable mutations from this menu can be mixed in various combinations to fine-tune phenotypes. This also will make it possible to modify vaccine virus to accommodate antigenic drift in circulating virus strains.

A second important use will be to explore new possibilities for improving live attenuated vaccine viruses. It is likely that new types of attenuating mutations can be developed. The level of immunity associated with natural infection might be improved quantitatively or qualitatively by modifying the virus by the inclusion of cytokine genes or additional T cell epitopes, by ablation of possible epitopes associated with reactogenicity, or by other manipulations. A third important use will be for the exploration of the role of individual viral genes in virus replication and pathogenesis.

Development of a Live Attenuated Parainfluenza Virus Type 3 (PIV3) Vaccine. One of the important goals of the laboratory is the development of a live attenuated vaccine to prevent the serious lower respiratory tract disease caused by PIV3, which accounts for about 11% of hospitalization of infants and young children for severe respiratory tract disease. The laboratory is pursuing two strategies to achieve this goal. The first strategy involves the use of conventional techniques to produce a live attenuated virus vaccine. Two types of live virus vaccines have been developed, the cold-passaged (*cp*) human PIV3 and a bovine PIV3 (BPIV3) that is antigenically related to human PIV3. The second strategy involves the use of molecular techniques to generate infectious virus from cloned genomic material.

Two live attenuated PIV3 candidate vaccines are in Phase 1-2 trials in infants and children, namely the cold-passage 45 mutant (*cp45*) of the JS wild type human PIV3 and a BPIV3. The two live virus vaccine candidates were shown to be safe, infectious, immunogenic, and genetically stable in seronegative infants and young children. In addition, the *cp45* mutant replicates to very high titer in simian Vero cells grown on microcarriers. The Master Seed stock of the *cp45* mutant maintains its temperature sensitive (*ts*), cold-adapted (*ca*), and attenuation (hamsters) phenotypes despite its high level of replication in the Vero cell microcarrier culture. Thus, at this stage the large scale

production of the *cp45* mutant appears feasible. Importantly, LID is in the final stages of negotiating a CRADA with Lederle-Praxis for the commercial development of the *cp45* PIV3.

A complete cDNA copy of the JS strain of human PIV3 has been constructed with the intent of rescuing infectious virus from PIV3-infected cells transfected with RNA transcribed from this synthetic cDNA copy of the PIV3 genome. The full length cDNA has the exact coding sequence of the wild type virus except for engineered mutations introduced by site-directed mutagenesis. These mutations were introduced to permit the virus rescued from the cDNA to escape neutralization by monoclonal antibodies that neutralize the infectivity of the helper PIV3. In addition, mutations that mark the DNA have been introduced to permit unequivocal identification of rescued virus. An alternative strategy similar to the plasmid-based system used successfully with RSV, was also pursued. This involved modifying the full length PIV3 cDNA so that it could be transcribed intracellularly into the full length genome that could be rescued into infectious virus. Both the helper virus-based rescue system and the plasmid-based rescue system are being used currently in attempts to recover infectious virus from the full length PIV3 cDNA.

New Strategy for Construction of Attenuated Mutants of Influenza A Virus for Use in a Live Virus Vaccine. During the past two years a new strategy was developed for the construction of live attenuated influenza A virus vaccine strains in which attenuating mutations were introduced into a cDNA copy of the PB2 gene (that encodes a protein of the viral polymerase complex) by site-directed mutagenesis after which the mutant PB2 gene (in the form of full-length RNA transcripts of cDNA) was rescued into an infectious virus. This year these initial findings were extended by demonstrating the feasibility of introducing a putative *ts* mutation into the PB2 gene at amino acid (aa) residue 112, 265, 556, or 658. Analysis of the resulting mutants indicated that each mutation indeed specified both the *ts* and attenuation (*att*) phenotypes. Viable mutants were then constructed that had two or three of these *ts* mutations in the PB2 gene of the influenza A/AA/6/60 virus. The AA mutant PB2 transfectants that possessed two or three PB2 *ts* mutations were highly attenuated and genetically stable in rodents and yet were able to induce significant protection against challenge with wild type virus. Sequential addition of single *ts* mutations into the PB2 gene brought about a step-wise increase in both temperature sensitivity and attenuation of the resulting transfectant virus. Such a PB2 gene bearing multiple mutations could be used alone, or in conjunction with another attenuating gene, to attenuate new epidemic influenza A viruses as they emerge in nature. The PB2 gene offers a distinct advantage over viral surface glycoprotein genes, such as the hemagglutinin or neuraminidase, as a site for one or more attenuating mutations because it occupies an internal location in the virion and hence plays a minor role if any in inducing immunity to infection. This means that the PB2 gene (or other internal genes) can be transferred from an attenuated donor virus to any new influenza A virus by gene reassortment without compromising the immunogenicity of the new virus. Formulation and validation of this new strategy ushers in a new era in the development of live attenuated vaccines for influenza A virus. For the first time defined multiple attenuating mutations have been introduced by site-directed mutagenesis into a viral gene encoding an internal protein that can be readily transferred to new epidemic variants of the virus to achieve the desired optimal balance between attenuation and immunogenicity required of a safe and effective live influenza A virus vaccine.

HEPATITIS VIRUSES SECTION

FY95 was also a year of considerable achievement for the Hepatitis Viruses Section. The first inactivated hepatitis A virus (HAV) vaccine to be licensed in Europe and the US was the product of a collaborative effort involving the Section and Smith-Kline Beecham RIT Inc. The virus strain used in the vaccine was recovered and characterized by scientists in the Section. Subsequently, it was adapted to grow more efficiently in cell culture by them. Later the cell culture-adapted mutant of HAV and a series of patents that specify this virus and its properties were licensed to Smith-Kline Beecham RIT who developed commercial procedures for scale-up and achieved clinical realization of the product. Extensive clinical trials demonstrated the safety and protective efficacy of the vaccine.

Search for New Hepatitis Viruses. One of the goals of the Section is to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A subsequent long-term objective is the development of passive and active immunoprophylaxis for these human pathogens.

Evidence for the existence of an additional water-borne hepatitis virus was obtained in FY95 during sero-epidemiologic studies of hepatitis in India and Saudi Arabia. From 50-100% of hepatitis cases in sixteen epidemics of water-borne hepatitis were caused by hepatitis E virus (HEV) but one water-borne epidemic was not caused by either HAV or HEV. Similarly, in Saudi Arabia, 13.4% of acute hepatitis in adults could not be diagnosed as hepatitis A through E and appeared to be transmitted by nonparenteral means.

Hepatitis B Virus (HBV). Scientists in the Section continued their characterization of a putative neutralization escape mutant of HBV that emerged in individuals who were vaccinated against hepatitis B. Two licensed vaccines were investigated for their ability to protect chimpanzees against challenge with the surface antigen (S) gene mutant. Both recombinant vaccines provided protection against challenge with the mutant virus, suggesting that properly vaccinated individuals are not at risk of infection by the S gene mutant virus.

In addition, a bi-directional promoter complex was identified within the X gene of HBV, further emphasizing the complex genetic organization of this virus. Since the relative importance of viral and host factors in fulminant hepatitis B has not been established, the sequences of HBV strains recovered from patients with fulminant hepatitis B are being analyzed and compared with published sequences of HBV strains recovered from other fulminant cases to determine if there are viral sequences uniquely associated with severe disease. Strains of HBV of particular interest (such as from cases of fulminant hepatitis) are being subjected to a new PCR amplification procedure that amplifies virtually the entire genome permitting recovery of infectious cDNA. The amplified genomes will be sequenced and, possibly, biologically amplified and characterized in chimpanzees. Long term follow up of the NIAID's 1975 plasma-derived vaccine, which was the first successful subunit vaccine developed, has continued. The vaccine continues to be highly efficacious five years after administration to infants.

Woodchuck Hepatitis Virus (WHV). WHV is taxonomically and serologically related to HBV. Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus,

WHV infection of woodchucks provides a relevant and convenient model for achieving an understanding of HBV infection of humans. Several lines of research were pursued. First, it was shown that the WHV X gene is very sensitive to alterations and is essential for viral replication in animal transfection experiments. Second, fine mapping of the X transcript promoter identified the essential nucleotides in this *cis*-acting element. Third, characterization of the bi-directional promoter of WHV has been extended. Fourth, two WHV isolates were shown to induce surface antigenemia and liver tumors in woodchucks at significantly different rates; this last finding has important implications for understanding the molecular mechanisms involved in viral replication and in the oncogenic potential of hepadnaviruses. Fifth, an *in vitro* cell culture system for transfection of WHV was developed. Sixth, the role of the capsid protein in viral replication was studied. Seventh, the role of the envelope proteins in viral replication was examined.

Hepatitis C Virus (HCV). HCV appears to be the major current etiological agent of transfusion-related non-A, non-B hepatitis. The HCV genome is a linear, positive-stranded RNA molecule of approximately 9,500 nucleotides and encodes a polyprotein of about 3,000 amino acids. Several stretches of amino acids in the HCV polyprotein share significant similarity with flavivirus and pestivirus proteins. Therefore, HCV is considered to be distantly related to these virus groups. The goal of this project was to increase our understanding of the molecular biology of this important human pathogen.

The virus contains a positive polarity, single-stranded RNA genome with 5' and 3' noncoding (NC) regions. The core (C), envelope 1 (E1) and envelope 2 (E2) proteins are encoded at the 5' terminus and the nonstructural proteins are encoded at the 3' terminus of the single open reading frame of the genome. The genetic heterogeneity of the HCV genome, especially in the genes encoding the envelope proteins, is similar to the heterogeneity seen in the envelope gene of human immunodeficiency viruses. Such a finding bodes ill for attempts at vaccine development.

Last year the 5' noncoding (NC) and envelope 1 (E1) gene sequences of the HCV RNA genome derived from 52 individuals from twelve countries were determined. These HCV isolates were found to comprise at least six major genetic groups and at least twelve minor genotypes. Subsequently, the entire core (C) gene was amplified using reverse transcription and polymerase chain reaction (RT-PCR). Nucleotide sequence analysis indicated that the gene was exactly 573 nucleotides in length in all 52 HCV isolates, with an amino terminal start codon and no in-frame stop codons. This represented the first report of C gene sequences of seven previously identified genotypes as well as two new genotypes. The phylogenetic analysis of the C gene sequences (which are the most conserved gene sequences in the HCV genome) was in agreement with that of the more viable E1 gene sequences and highlighted a major taxonomic division among isolates in which genotype 2 was distinct from other genotypes. The predicted C proteins of the different HCV genotypes shared the following features: (i) high content of proline residues, (ii) high content of arginine and lysine residues located primarily in three domains with ten such residues invariant in the region bounded by amino acid positions 39-62, (iii) a cluster of five conserved tryptophan residues, (iv) two nuclear localization signals and a DNA-binding motif, (v) a potential phosphorylation site with a serine-proline motif, and (vi) three conserved hydrophilic domains that have been shown to contain immunogenic epitopes. The results of this study have implications for the taxonomy of HCV and for the diagnosis, prevention and therapy of HCV infections.

Most recently, the first hypervariable region (HVR1) of the envelope 2 gene has been sequenced and analyzed. This region, which is situated at the 5' end of the E2 gene, comprises approximately thirty amino acids and is the single most heterogeneous region of the entire genome.

It is thought to contain one or more B cell epitopes and to be a significant target of the immune system. Surprisingly, despite its heterogeneity, the HVR1 contains certain conserved features. For instance, several amino acids are invariant in all of the strains of HCV examined. In addition, an underlying pattern of hydrophilicity appears to be maintained in all isolates.

Hepatitis C virus has been shown to infect several cell types *in vitro*, most notably human continuous T cell lines. Such infection, although not useful for growing the virus, has been utilized to detect neutralizing antibodies and the localization of a neutralization epitope on the virus. Our collaborator, Dr. Yohko Shimizu (a former visiting scientist in the section), has used several lines of modified T cells to demonstrate most of the components of the replicative cycle of HCV, including attachment, penetration, uncoating, transcription, translation and at least partial assembly. She has also demonstrated that attachment of the virus to these cells can be prevented by specific antibody. This observation has been used to develop a neutralization assay which was employed to demonstrate the appearance and disappearance of neutralizing antibodies over time in chronically infected patients.

Another *in vitro* neutralization assay was developed which measured neutralization of infectivity of HCV incubated with serum prior to inoculation of the virus-serum mixture into a chimpanzee. In this assay failure of a chimpanzee inoculated with an antigen-antibody mixture to become infected with HCV was interpreted as evidence for presence of neutralizing antibodies in the test serum. In general, the results obtained by Shimizu *in vitro* were confirmed by the newly developed *in vivo* system, thereby demonstrating the relevance of the cell culture-based neutralization assay. Both the cell culture assay and the *in vitro* / *in vivo* chimpanzee assay have been used to begin the mapping of neutralization epitopes. In collaboration with Dr. Teresa Cabezon, it was demonstrated that hyperimmune rabbit serum prepared against synthetic peptides based upon the sequence of the HVR1 can neutralize HCV. Dr. Shimizu is currently mapping the epitope within the HVR1. This information will be important in determining the extent of serologic heterogeneity of HCV, an important first step in practical vaccine development.

Neutralization of HCV *in vitro* was attempted with plasma of a chronically infected patient and the presence or absence of residual infectivity was evaluated by inoculation of eight seronegative chimpanzees. The source of HCV was plasma obtained from the patient during the acute phase of post-transfusion hepatitis, which had previously been titered for infectivity in chimpanzees. Neutralization was achieved with plasma obtained from the same patient two years after the onset of primary infection, but not with plasma obtained eleven years later, although both plasmas contained antibodies against nonstructural and structural (including envelope) HCV proteins. Analysis of sequential viral isolates obtained from the same patient revealed a significant genetic divergence as early as two years after infection. This evidence, together with the divergent sequences of HCV recovered from the chimpanzees that received the same inoculum, confirms that HCV is present *in vivo* as a quasispecies. These observations provide the first experimental evidence *in vivo* that HCV infection elicits a neutralizing antibody response in humans, but suggest that such antibodies are isolate-specific. This emphasizes the difficulties that lie ahead for the development of a broadly reactive vaccine against HCV.

More recently, additional chimpanzees were employed to extend the above studies. Rabbit preimmune and hyperimmune sera, prepared against a synthetic peptide representing the HVR1 sequence of HCV recovered from the patient described above during the acute phase of his infection, were studied for neutralizing activity. The preimmune serum did not protect the chimpanzee against hepatitis C but one of two chimpanzees inoculated with rabbit hyperimmune serum plus HCV was protected. Virus recovered from the unprotected chimpanzee was shown to have a different

sequence in the HVR1 region, indicating that a neutralization escape mutant virus, probably present as part of the quasispecies population within the inoculum, emerged in this animal. Indeed, an extensive sequence analysis of more than 100 molecular clones of HCV derived from the inoculum demonstrated twenty unique sequences comprising the quasispecies of this HCV inoculum. Thus, control of hepatitis C by vaccination will be difficult unless more broadly reactive immunizing antigens can be prepared or more broadly protective viral epitopes can be identified.

Hepatitis A Virus. HAV is a picornavirus with a single-stranded RNA genome of approximately 7,500 nucleotides. The wild-type strain of HAV grows poorly in cell-culture, generally is not cytopathic, and virus yields are low. A cell-culture adapted mutant (HAV/7) has been selected which grows significantly more efficiently in cell-culture and which is attenuated for marmosets and chimpanzees. The objectives of this project were to determine the genetic basis for virulence and adaptation to cell culture of HAV in order to develop a strain of HAV suitable for use as an attenuated vaccine. During the course of these studies several advances in our understanding of HAV were made.

In an effort to increase replicative capacity in cell culture, chimeric viruses were constructed from two or more HAV strains including a virulent human strain, an attenuated strain, a vaccine strain, a cytopathic strain, and a simian strain. The P2 region from a cytopathic strain of HAV was shown to confer the large focus phenotype but not the lytic phenotype of the cytopathic virus. Both halves of the P2 region were involved but not all of the mutations were required. Attenuated HAV (HAV/7) chimeras containing the 2C gene of the simian virus grew less efficiently than did HAV/7 but intragenic 2C chimeras grew at an intermediate level. The first phenotype linked to the 2A gene was identified when it was observed that an engineered point mutation in 2A caused accumulation of viral capsids in the nucleus.

Chimeric viruses were constructed and assayed for the purpose of identifying virulence genes. The 2A gene was identified as a second major determinant of attenuation of HAV/7 for marmosets and evidence was obtained that mutations in the 2A and 2C genes are almost totally responsible for the attenuation of this virus.

Hepatitis E Virus. Epidemics of enterically transmitted non-A, non-B hepatitis (hepatitis E) have been reported in Asia, Africa, and North America. Similar cases of sporadic hepatitis, presumed to be hepatitis E, account for up to 90% of reported hepatitis in countries where hepatitis E is endemic. HEV has been implicated in fulminant hepatitis of pregnancy, a disease with a high fatality rate. That a viral agent was responsible for hepatitis E epidemics was first shown in 1983. On the basis of electron microscopy and molecular characterization it has been proposed that HEV belongs to the calicivirus family, but in fact it may belong to a new family of viruses.

The goals of our research in this area are to characterize the newly identified HEV with respect to its molecular and genetic properties, to determine the extent and pattern of its involvement in enterically transmitted hepatitis, and to develop a vaccine which prevents hepatitis E. A seroepidemiologic study revealed that HEV is a major cause of acute viral hepatitis in Saudi Arabia, however, the cases occur mainly in expatriates and not in Saudis. In addition, it was observed that pregnant rhesus monkeys did not develop more severe disease than nonpregnant monkeys. Rhesus mothers infected with HEV did not transmit the virus to their fetus *in utero*.

The ORF-3 protein of HEV was tentatively identified as an RNA-binding protein. Studies of active immunoprophylaxis were also actively pursued. Last year it was shown that baculovirus recombinant-expressed HEV ORF2 (viral capsid) protein induced resistance in monkeys to

subsequent challenge with virulent HEV. These efforts are being extended in order to determine the optimal protocol for immunization against hepatitis E.

MOLECULAR VIRAL BIOLOGY SECTION

Dengue viruses which are members of the Flaviviridae are transmitted to humans principally by *Aedes* mosquitos, i.e., *A. albopictus* and *A. aegypti*, and are responsible for dengue outbreaks and epidemics throughout the subtropical and tropical regions of the world. Dengue disease varies in severity ranging from mild febrile illness to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Worldwide, the dengue viruses cause more disease in humans than any of the other arthropod-borne flaviviruses.

Mouse-Adapted Dengue Virus Mutants: Mouse Neurovirulence and Attenuation for Humans. Shortly after the first strains of dengue virus (DEN) were recovered fifty years ago, serial passage of DEN serotype 1 or 2 in mouse brain was shown to rapidly select for neurovirulent mutants that concomitantly exhibited significant attenuation for humans. This rapid coordinate acquisition of mouse neurovirulence and attenuation for humans suggested that the genetic basis of these two phenotypes might be related. Scientists in the Section had previously employed full-length infectious DEN4 cDNA to construct viable intertypic dengue chimeras that contained the genes for the structural C-PreM-E proteins or only the PreM-E proteins of another dengue virus serotype substituting for the corresponding genes of the non-neurovirulent DEN4. Analysis of such a chimera containing the genes for the structural proteins of the mouse neurovirulent mutant of the DEN2 NGC strain identified the structural genes as the site of the mouse neurovirulence mutations. Subsequently, insertion of each of the mutations identified in these genes, either singly or in combination, into a non-neurovirulent parental DEN2 NGC/DEN4 chimera identified a Glu₄₀₆ to Lys substitution in the envelope glycoprotein (E) as responsible for the acquisition of dengue type 2 virus mouse neurovirulence. This observation suggested that it might be possible to create a similar attenuating mutation in dengue viruses of other serotype as part of a general strategy for vaccine development. The usefulness of this strategy was evaluated in a preliminary experiment in which a viable DEN3/DEN4 chimera was constructed that contained DEN3 C-PreM-E genes and expressed DEN3 antigenic specificity. A Glu₄₀₆Lys substitution homologous to that present in the DEN2 mouse neurovirulent mutant was then introduced into the chimera and intracerebral inoculation of suckling mice revealed that the mutant was neurovirulent, whereas its chimeric parent or parental DEN3 was not. These encouraging results suggest that this strategy might be successful for construction of attenuated dengue mutants for use in a live virus vaccine if subsequent studies demonstrate that the same mutation or mutations are responsible for both mouse neurovirulence and attenuation for humans. However, it should be noted that the Glu₄₀₆Lys mutation in E is only one of the mutations that have shown to be responsible for dengue virus mouse neurovirulence. For example, other mutations in E such as Thr₄₃₄Leu or Phe₆₈₀Leu are responsible for dengue type 4 virus mouse neurovirulence. If these mouse neurovirulence mutations also prove to be responsible for human attenuation, this polymorphism actually creates new opportunities because it might be possible to fine-tune the level of attenuation of a mutant for humans by combining different mutations in a single vaccine construct.

Restriction of Growth of Neurovirulent DEN4 Mutant in Simian Cell Culture. Previously a neurovirulent mutant of DEN4 (strain H241) was also selected by serial intracerebral passage in mice. Unexpectedly, the DEN4 neurovirulent (N) mutant was observed to replicate less efficiently than its DEN4 parent (P) in simian LLC-MK₂ cells. An intratypic DEN4 chimera that derived its C-PreM-E structural protein genes from DEN4 N also exhibited marked restriction of growth in simian LLC-MK₂ cells. Protein analysis indicated that DEN4 E, PreM, M and C were detected in a virion preparation of DEN4 P or its derived intratypic chimera in which the structural genes of DEN4 P were substituted into the DEN4 cDNA clone of another non-neurovirulent strain. On the other hand, a virion preparation of DEN4 N or its derived chimera contained E, PreM and C, but not M. This suggested that cleavage of PreM to M was defective for DEN4 N and the genetic locus or loci for the defect mapped within the C-PreM-E genes. There were six amino acid differences in the structural protein gene region between DEN4 P and DEN4 N: one in C, two in PreM and three in E. To identify mutations responsible for defective PreM cleavage, eight chimeric mutants were constructed that contained one or more amino acid substitutions that are present in the mutant C, PreM or E. Only mutant DEN4(P, S456), into which all three amino acid substitutions in E were introduced, exhibited the PreM cleavage defect. Interestingly, chimeric mutants which contained only the two mutations in PreM processed PreM normally. This supports the previous suggestion that PreM interacts with E during virus maturation and changes in the latter can have a profound effect on processing of the former protein. The implications of this observation to development of live attenuated dengue virus vaccines are being pursued.

Deletion Analysis of the 3' and 5' Non-Coding Regions of the Dengue Virus Genome. Success in cloning a full-length DEN4 cDNA which can be used to produce infectious RNA transcripts has created opportunities for detailed molecular analysis of the positive strand RNA genome and for development of new dengue vaccine strategies. A panel of viable 3' noncoding (NC) deletion mutants was recovered from RNA transfected simian LLC-MK₂ cells. Most 3'NC deletion mutants produced plaques that were slow to develop on simian LLC-MK₂ cells. Interestingly, mutant 3'd 303-183 produced small plaques on mosquito C6/36 cells but grew to a high titer similar to that of wild type virus in simian LLC-MK₂ cells. Mutant 3'd 303-183 and four other 3'd mutants that were moderately restricted in growth in simian cell culture were then tested for infectivity and immunogenicity in rhesus monkeys. Mutant 3'd 303-183 induced a high level antibody response similar to that stimulated by wild type virus, whereas other mutants induced a low to moderate level of antibodies as measured by radio-immunoprecipitation and virus neutralization. Unexpectedly, the results of a later preclinical trial in monkeys showed that three 3' NC deletion mutants selected as candidate vaccine strains and the wild type DEN4 control, all prepared anew from cDNA and produced in simian FRhL cells under GMP conditions, failed to elicit an antibody response. Experiments in progress indicate that propagation of virus in simian FRhL cells (that are approved for use in experimental vaccines destined for study in humans) selected for virus with reduced infectivity for monkeys.

In other studies, DEN4 cDNA was used to engineer deletions in the 5' NC region for functional analysis and for isolation of mutants that might show promise as candidate live vaccine strains. Several 5' NC deletion mutants that exhibited low to moderate efficiency of translation *in vitro* produced small plaques and exhibited reduced growth in simian cell culture. Among the mutations tested, deletion of nt 82-87 in the 5'NC region most severely reduced translation efficiency. Nevertheless, an infectious virus was recovered from simian LLC-MK₂ cells transfected with the RNA transcripts of mutant 5'd 82-87. The progeny virus produced small plaques on simian

LLC-MK₂ cells and grew to low titer in these cells. Unlike wild type DEN4 or other 5' NC deletion mutants, mutant 5'd 82-87 failed to produce plaques on mosquito C6/36 cells and was also replication-defective in *A. aegypti* or *A. albopictus* following intrathoracic inoculation.

Intertypic Dengue Virus Chimeras for Use in Immunization. The current strategy for dengue immunization favors the use of a vaccine containing four serotypes. We previously employed full-length DEN4 cDNA to construct a viable intertypic dengue virus of type 1 or type 2 antigenic specificity that contained the capsid-premembrane-envelope (C-PreM-E) or only the PreM-E genes of DEN1 or DEN2 substituting for the corresponding DEN4 genes. Chimeras DEN1/DEN4 and DEN2/DEN4 which express the seven nonstructural proteins of DEN4 and the structural proteins of DEN1 or DEN2, and therefore the antigenicity of type 1 or type 2, were used to infect rhesus monkeys. Control groups were inoculated with parental DEN1, DEN2, or cDNA-derived DEN4. Three of four monkeys immunized with DEN1/DEN4 developed neutralizing antibodies against DEN1, and were protected against subsequent DEN1 challenge. All four monkeys immunized with DEN2/DEN4 developed neutralizing antibodies against DEN2 and were protected against subsequent DEN2 challenge. DEN1- or DEN2- immunized monkeys in the control groups were protected against homologous challenge, but DEN4-immunized animals became viremic on cross-challenge with DEN1 or DEN2. In a second experiment, eight monkeys were immunized with an equal mixture of DEN1/DEN4 and DEN2/DEN4. Each animal developed neutralizing antibodies against both DEN1 and DEN2, and was protected against DEN1 or DEN2 challenge. Chimeric dengue viruses such as those evaluated in this study could be used to express their serotype-specific antigens in a tetravalent human vaccine.

Tick-Borne Encephalitis Viruses (TBEV): Molecular Studies and Construction of Attenuated TBEV/DEN4 Chimeras. Studies involving tick-borne encephalitis virus (TBEV) represent a logical extension of ongoing studies of the dengue viruses. Dengue viruses and members of the TBEV complex both belong to the flavivirus genus of the Flaviviridae. TBEV causes serious disease with high mortality throughout Russia, India, Scandinavia and Central Europe. In addition, a virulent member of the TBE complex, Powassan virus, is present in Canada and the Eastern and Western US. Twenty cases of Powassan encephalitis have been reported in the US and Canada but more ominous is the wide distribution of the virus in small mammals.

Previously, viable chimeric flaviviruses were constructed that contained TBEV structural protein CME or ME genes with the remaining genes derived from DEN4. When tested by the intracerebral route, the ME chimera retained the neurovirulence for mice of its TBEV parent from which its M and E genes were derived, but when tested by parenteral inoculation, it lacked the peripheral invasiveness of TBEV. The ME chimera was subjected to mutational analysis in an attempt to reduce or ablate neurovirulence manifest when virus is inoculated directly into the brain. Three distinct mutations were independently associated with marked reduction of mouse neurovirulence. Significantly, parenteral inoculation of these attenuated mutants induced complete resistance in mice to fatal encephalitis caused by subsequent challenge with the highly neurovirulent ME chimera. These observations suggest a new strategy for the development of a live attenuated TBEV vaccine.

Unlike the highly virulent TBEV, the wild type Langat virus (TP21 strain), the least virulent of all TBEV-complex flaviviruses, has low encephalitogenic potential and low peripheral virulence and has not been reported to be associated with any human disease. In an attempt to identify the molecular basis for attenuation of TBEV-complex viruses, the sequence of the genome of wild type

Langat virus (LGT) virus strain TP21 and a more attenuated strain derived from it (strain E5) were determined. Among the tick-borne flaviviruses, the LGT genome differs in length of its 3' noncoding region compared to the genome of the Russian TBEV or Powassan virus (a TBE virus of North America). Analysis of TP21 and E5 genome sequences revealed six amino acid differences in the polyprotein, one of which was a substitution Asn > Asp in position 387 of E protein, that is probably responsible for the further attenuation exhibited by the E5 virus. Potential attenuating mutations which were identified by sequence analysis of the LGT strains will be introduced into chimeric LGT/DEN4 and TBEV/DEN4 genomes and viable progeny viruses will be analyzed for level of attenuation and immunogenicity.

EPIDEMIOLOGY SECTION

Rotaviruses are the single most important etiological agents of severe diarrheal disease in infants and young children in both the developed and developing countries. These viruses are estimated to cause 30 to 50% of such illness in the United States as well as world-wide. The consequences of such illness in developing countries are particularly grim because severe rotavirus disease in these regions is estimated to cause ~900,000 deaths each year. Thus, the need for an effective vaccine able to prevent severe rotavirus disease is clear.

Live Attenuated Quadrivalent Rhesus Rotavirus Vaccine. Scientists in the Epidemiology Section have taken a "Jennerian" approach to vaccine development. This involves the use of a live animal virus that is: (i) attenuated for humans, (ii) antigenically related to the human virus to be immunized against, and (iii) able to induce protective immunity against the human virus. Initial studies were performed with a simian rotavirus, rhesus rotavirus (RRV), that is closely related antigenically to human rotavirus serotype 3. Impressive protective effect was observed against serotype 3 human rotavirus disease but efficacy was variable against other rotavirus serotypes associated with disease, *i.e.*, 1, 2 and 4. We, therefore, developed a modified "Jennerian" approach that involved formulation of a quadrivalent rotavirus vaccine containing: (i) rhesus rotavirus (RRV) (VP7 serotype 3), and (ii) three human rotavirus-RRV reassortants, each possessing ten RRV genes and a single human rotavirus gene that encodes VP7 (the major protective antigen) of serotype 1, 2, or 4.

FY 1995 has been a banner year for this research program because the results of two recently completed studies in the United States indicate that the quadrivalent formulation provided a high level of protective efficacy against severe rotavirus diarrheal disease. Under the auspices of our licensee, Wyeth-Ayerst Research, a multi-center (23 sites), three cell prospective, double-masked, placebo-controlled efficacy trial covering a period of two rotavirus seasons was performed. Protective efficacy against serious rotavirus disease was 82%. In addition there was a 78% reduction in need for medical visits for rotavirus disease. These encouraging observations were confirmed and expanded during a second large multi-center trial. Quadrivalent vaccine administered at a ten-fold higher dosage was 80% protective against severe rotavirus diarrheal disease. Most important was the observation that the quadrivalent vaccine was 100% effective in preventing dehydrating illness, the most severe form of rotavirus diarrhea.

Second Generation Live Attenuated Rotavirus Vaccines. Group A rotaviruses possess two outer capsid proteins that function as independent neutralization antigens, namely VP4 (encoded by genome segment 4) and VP7 (encoded by genome segment 7, 8, or 9 depending on the strain). Although initially VP7 was thought to be the dominant neutralization antigen, recent studies have shown that VP4 is as effective as VP7 in inducing neutralizing antibodies following infection of experimental animals or susceptible infants or young children. Also, antibodies to VP4 or VP7 are independently associated with resistance of gnotobiotic piglets to experimental challenge with virulent rotavirus. However, VP7 is the only relevant rotavirus protective antigen present in candidate vaccines, such as the quadrivalent RRV vaccine, that are currently being evaluated for protective efficacy in humans, because these vaccines contain the VP4 of an animal rotavirus that is not related antigenically to the VP4 of any of the clinically important human rotaviruses.

Although the quadrivalent RRV provides ~80% protection against severe rotavirus disease and 100% protection against the most severe rotavirus disease (*i.e.*, dehydrating diarrhea), it might be possible to improve overall efficacy by incorporating both protective antigens (*i.e.*, VP4 and VP7) of the clinically most important rotaviruses. In an attempt to achieve this goal we pursued another approach to vaccine development in which mutants of human rotaviruses bearing both human rotavirus VP4 and VP7 were generated. The strategy used entailed the sequential selection of mutants that were able to grow efficiently at the suboptimal temperature of 30°C, 28°C, or 26°C. Mutants selected in this manner were both cold-adapted (*ca*) and temperature-sensitive (*ts*). Previously, this strategy has been employed successfully to select various attenuated candidate human viral vaccine strains for several respiratory tract viral pathogens.

Similar *ca*, *ts* mutants were derived from human rotaviruses of major epidemiologic importance thus providing broad antigenic coverage for: (i) the clinically most important human rotavirus VP4, *i.e.*, serotype 1A and (ii) each of the four clinically most important rotavirus VP7 serotypes, *i.e.*, 1, 2, 3, and 4) that account for more than 95% of human rotaviruses recovered from patients with diarrhea. In the case of each of the four VP7 serotypes, mutants selected at 30°C, 28°C, or 26°C exhibited a wide range of cold-adaptation and temperature sensitivity suggesting that these mutants encompassed a spectrum of attenuation for susceptible infants. This should increase the likelihood that satisfactory vaccine strains had been recovered. One of these candidate vaccine mutants (strain D, VP4 serotype 1A and VP7 serotype 1) is currently under clinical evaluation.

One or more of the *ca* mutants might prove useful in supplementing the quadrivalent RRV vaccine by providing the clinically relevant VP4 1A antigen that is present on ~85% of human rotaviruses recovered from patients with diarrheal disease. Alternatively, a quadrivalent *ca* vaccine might exceed the quadrivalent RRV vaccine in efficacy.

Human Calicivirus Gastrointestinal Pathogens. The Norwalk and Norwalk-like human caliciviruses are major etiologic agents of epidemic nonbacterial gastroenteritis. A serious obstacle to the study of these viruses has been our inability to grow them in cell culture. Nonetheless, there is a compelling need to identify the extent of antigenic polymorphism among the Norwalk-like viruses, define the genetic basis of antigenic diversity and chart the epidemiologic importance of these human pathogens. Progress was made in each of these research areas during FY95. Baculovirus-expressed, self-assembled virus-like particles (VLPs) that contain the viral capsid protein (encoded by open reading frame 2 [ORF2]) of the DS395 Desert Shield virus, the TV24 Toronto virus, and the Hawaii virus (HV) were used to develop diagnostic assays and serologic reagents to examine antigenic relationships among these and other calicivirus reference strains. Relationships established with these recombinant protein-based assays should provide a framework

for the development of a provisional system for classification of human calicivirus serotypes that will ultimately be refined when neutralization assays can be performed. In addition, recombinant calicivirus proteins are being used to study basic biological features of the virus.

Because of our continued inability to cultivate any of the pathogenic human caliciviruses in cell culture, we have used a cultivatable feline calicivirus, URB, as a surrogate for the study of calicivirus biology and replication. The sequence of the 7.7 Kb RNA genome of the URB strain was determined from cloned cDNA fragments. These sequences were then utilized to construct a full-length cDNA copy of the genome that was positioned in a plasmid downstream from the T7 RNA polymerase promoter. Full-length synthetic RNA transcripts prepared from this clone were infectious when transfected into feline kidney cells, thus providing us with an opportunity to gain new insights into the replication of this family of viruses and their relationship to other positive-strand RNA viruses. Currently, the infectious feline calicivirus cDNA clone (serving as a surrogate for Norwalk virus) is being used to study receptor binding, infectivity of viral RNA, position and product of various coding regions in the viral genome, protein processing, viral pathogenesis, and the mechanisms responsible for host cell restriction in cell culture.

IMMUNODEFICIENCY VIRUSES SECTION

A crucial element in the development of effective therapeutic and prophylactic strategies for AIDS is an experimental animal model in which pathogenesis of infection by an immunodeficiency virus native to that host species parallels that of the human virus in human disease. Simian immunodeficiency virus (SIV) infection of macaques satisfies these criteria and is therefore a suitable surrogate for human AIDS. Notably, SIV induces an immunodeficiency syndrome in infected macaques that is remarkably similar to human AIDS. In addition, SIV utilizes the CD4 molecule as a receptor, and the pathogenesis of disease appears similar. This animal model system has proved to be very useful for the detailed study of pathogenesis and viral determinants of disease under conditions that can not be duplicated in human AIDS. These studies will allow us to determine how the primate lentiviruses destroy the immune system of their hosts, and this understanding should facilitate the development of more rational therapeutic antiviral strategies.

A Pathogenic SIVagm Strain Isolated from an African Green Monkey (AGM). SIVagm is generally considered to be minimally pathogenic both for its natural host and macaques. Despite this widely held view, scientists in the Section isolated a strain of SIV (SIVagm9063) from AGM which induced immunodeficiency in experimentally infected pig-tailed (PT) macaques but not in AGM or rhesus monkeys (RH). Importantly, virus derived from a full-length infectious molecular clone of this isolate also induced AIDS in PT macaques, but not in RH macaques or AGM, the latter being the natural host. Significant differences in virus load were observed during primary infection of PT macaques, RH macaques or AGM with this cloned virus. A high burst of virus expression was observed during the early stage of infection of PT macaques, whereas virus levels were significantly lower in infected RH or AGM. These data suggest that species-specific virulence is associated with the extent of viral replication *in vivo*. More extensive comparison of this virus in monkeys which are resistant (AGM and RH) or permissive (PT macaques) as well as more detailed molecular analysis of the virus should shed additional light on viral as well as host determinants of virulence.

Recovery of an Antibody-Resistant Clone of SIVsm. SIVsmE543 clones were derived from a highly pathogenic isolate of SIV recovered from an immunodeficient rhesus macaque, E543. Similar to primary clinical isolates of HIV-1, this molecularly cloned virus was resistant to neutralization *in vitro* by serum of SIVsm infected monkeys that neutralized related laboratory strains of SIVsm with high efficiency. This molecular clone will be used to generate chimeras in which portions of the envelope glycoprotein of a neutralization sensitive clone (smH4) will be substituted in an effort to identify regions of the SIVsm E543 envelope that contribute to its antibody-resistant phenotype.

Factors Involved in Progression of Disease in SIV Infected Monkeys. Macaques which were infected with molecularly cloned SIVsm or SIVagm and which either developed AIDS or failed to exhibit evidence of progression to disease were compared for: (i) virus load; (ii) humoral immunity; and (iii) sequential genetic variation of SIV. In one study, six macaques were infected with SIVsm62d; two developed AIDS 1 to 1.4 years after infection, whereas the remaining four animals remained healthy. In general, virus load in plasma samples from the latter animals, as measured by QC-PCR, was relatively low, particularly after the primary phase of infection. Variation of SIV derived from sequential samples of peripheral blood mononuclear cells (PBMC) was minimal in these non-progressor animals and a moderate serum neutralizing antibody titer was attained. In contrast, virus load in the progressor monkeys remained high after the primary phase of infection and extensive variation within the envelope glycoprotein of virus derived from PMBC was noted by six to ten weeks post infection. In addition, these animals each developed a high titer of serum neutralizing antibodies. SIV expression, as measured by *in situ* hybridization, could not be detected in tissues of the two nonprogressors tested, whereas SIV-expressing lymphocytes were readily detected in tissues of the progressors. These data suggest a dominant role for viral replication in AIDS pathogenesis. Also, the extensive variation of SIV in PBMC of progressors suggest that one of the major elements driving viral variation is active, high level replication of the virus. A similar study of SIVagm9063-2 infected PT macaques has been initiated.

Tissue Tropism of SIV. Molecular clones of SIVsm derived from tissues of infected macaques were used to study the determinants of tropism and their role in pathogenicity. The determinants of PBMC and macrophage tropism were mapped to a region including, but not exclusive to the V3 loop analog of gp120. Naturally-occurring mutations at a highly variable residue within the V3 analog were crucial in determining tropism but residues C-terminal to the loop were also important. Variation in tropism for primary macaque cells was associated with marked differences in pathogenicity. Thus, virus (62D) that replicated in macaque PBMC and macrophages was most pathogenic, virus (62A) that replicated inefficiently in PBMC and not at all in macrophages induced a more transient infection, and virus (62B) that did not infect either of these primary cells did not infect macaques.

Sequence Variation of SIV in Different Tissues. Virus populations within lymphoid tissues, PBMC and brain of two SIV-infected macaques which had progressed to AIDS were examined by single stranded conformational polymorphism of PCR products of the envelope, followed by sequence analysis. Extensive heterogeneity of SIV was observed in lymphoid tissues while viral populations within the brain and PBMC were significantly more homogeneous. Sequence analysis revealed a distinct viral envelope sequence in infected brain. These viruses had a highly divergent V1 region (a hypervariable region) as well as substitutions in residues of the V3 analog previously implicated as playing a role in tissue tropism. In addition, a stop codon in the cytoplasmic domain

of envelope glycoprotein was observed in fourteen of twenty brain clones but not in any of the twenty PBMC clones tested, suggesting a role for envelope truncation in neurotropism.

Small Animal Model for Experimental HIV Infection. An attempt was made to identify a small experimental animal in which chronic HIV-1 infection could be induced. The availability of such a small animal model for HIV-1 might facilitate screening of experimental immunogens and antiviral compounds and allow us to evaluate their protective or therapeutic efficacy in a timely fashion before proceeding to studies in non-human primates. The first small experimental animal studied, the cotton rat, proved to be susceptible. Chronic infection was induced and could be passaged from animal to animal three times sequentially. Infection was not extensive but it was sufficient to be easily detected by PCR or by development of an HIV-1 antibody response.

Immunization Strategies that Fail to Prevent Infection but Nonetheless Beneficially Affect the Course of Disease. The SIV/macaque model of AIDS was used for the evaluation of potential vaccine strategies for control of human HIV disease. Emphasis was given to the evaluation of a recombinant of the highly attenuated vaccinia virus (modified vaccinia virus Ankara; MVA) that expressed the SIVsmH4 gag-pol and env (MVA-SIV). This recombinant was compared to a similar recombinant constructed from the much less attenuated Wyeth strain of vaccinia virus (Wyeth-SIV). The Wyeth vaccinia virus was a licensed product that had been used previously in large immunization campaigns for prevention of small pox. Four rhesus macaques per recombinant immunogen were immunized four times over a 46-week period. Whereas, the MVA-SIV boosted the serum SIV antibody response following each of three sequential inoculations, the Wyeth-SIV recombinant only boosted at the second immunization and antibody levels declined subsequently. Prior to challenge with cell-free SIV grown in simian cells, the recombinant SIV-immunized monkeys were boosted with whole psoralen-inactivated human cell culture-grown SIV (WI-SIV) administered without adjuvant. Although immunization did not prevent infection following intravenous challenge with uncloned SIVsm, the dynamics of virus replication were altered significantly. Three of the four MVA-SIV-vaccinees exhibited sustained suppression of viremia throughout the year following challenge. This pattern of reduced viremia was associated with maintenance of normal lymphocyte subsets and disease-free status, analogous to long term nonprogressors of HIV-1 infection. In contrast, three of four monkeys immunized with the Wyeth-vaccinia SIV recombinant and three of four monkeys of the naive control group developed AIDS by one year. These data suggest that immunization with MVA-SIV in conjunction with an WI-SIV boost beneficially modified the pathogenesis of subsequent SIV infection.

Two additional studies were initiated to dissect the contribution of MVA-SIV or a boost with inactivated SIV to the down modulation of virus load observed in the pilot study. In the first of these trials, macaques were boosted three times with MVA-SIV or nonrecombinant MVA (four macaques per group) and challenged with 50 monkey infectious dose₅₀ (MID₅₀) of cell-free SIVsmE660 grown in macaque PBMC. All of these animals became infected following challenge. At ten weeks post challenge the plasma virus load of the macaques immunized with MVA-SIV was approximately ten-fold lower than that of the majority of control monkeys. Two of the control macaques failed to develop a humoral response and appear to be progressing rapidly to AIDS. We will continue to monitor the clinical course, and virus load of this group of macaques but these preliminary results suggest that restriction of virus replication was less profound than in the previous trial. This observation suggests that the boost with inactivated SIV played a role in suppressing viral load during subsequent infection.

The second study involved sixteen macaques; eight were immunized with MVA-SIV as above and then boosted (twice) with WI-SIV plus MDP adjuvant and eight served as naive controls. Half of these animals were challenged with the cell-free cloned homologous SIVsmH4 virus (grown in macaque PBMC) and the other half with the more virulent cell-free uncloned SIVsmE660 stock (grown in macaque PBMC) used previously. As in prior studies, animals will be monitored for reduction of virus load and clinical outcome.

A group of five macaques that had been immunized with psoralen WI-SIV and shown to be protected from cell-free SIV challenge were also studied. Initially, it was thought that the observed protective effect of WI-SIV was virus-specific. However, later work by other scientists (and confirmed in LID) indicated that the vaccine virus had incorporated human cell surface antigens during its propagation in a human continuous cell line and as a consequence, resistance to infection by challenge virus, also grown in the same human continuous cell line, was mediated entirely or in part by antibodies to human cell surface antigens. During the past year we sought evidence that a virus-specific immune response also contributed to the observed efficacy of WI-SIV. The monkeys previously shown to be resistant to challenge with human cell culture-grown SIV were boosted with WI-SIV and then challenged with simian cell-associated SIV. The challenge stock (SIVsm/E543 PBMC) was generated by separation of PBMC from the blood of an immunodeficient rhesus macaque (E543) at the time of autopsy and macaques were inoculated with the virus suspension to confirm its pathogenicity and to determine its infectivity titer. PCR-derived clones of the envelope demonstrated 92% amino acid identity with the vaccine strain. Because the virus challenge in the current study was derived from simian PBMC, it is unlikely that the development of antibodies to human antigens present on WI-SIV would play a role in the observed response to challenge. Thus, the use of this simian cell-derived SIV challenge would nullify the protective effect of antibodies to human cell surface antigens and allow us to examine the role, if any, of virus-specific immunity. Each of the macaques became infected but the survival of immunized macaques was significantly longer ($p < 0.03$ in a Student's *t* test) than that of naive controls. Plasma viremia was not detected in any of the immunized animals, whereas, all naive animals had detectable SIV antigen in plasma at two weeks post challenge. These observations suggest the WI-SIV vaccine altered the course of infection beneficially by a mechanism independent of incorporation of human cell surface antigens into the vaccine virus.

Passive Immunotherapy of SIV Infection. A recent passive immunotherapy study performed in collaboration with Philip Johnson (Ohio State University) and Nancy Haigwood (Bristol-Myers Squibb) yielded the unexpected finding that passive transfer of simian IgG containing SIV neutralizing antibodies 24 hours after infection with SIV had a definite beneficial effect by preventing subsequent progression of disease. The SIV immune globulin was prepared from the pooled plasma of a long term surviving SIV-infected monkey collected seven years post-infection. This observation suggests that SIV antibodies might find a use in the therapy of human AIDS. However, simian SIV-immune globulin is not a standard product and hence it would be desirable to prepare a well characterized set of SIV monoclonal antibodies that exhibit high neutralizing activity for SIV and that could be produced in large quantity whenever needed. The availability of such antibodies would allow us to characterize certain immunological determinants of resistance to infection in a precise and quantitative manner. Fortunately, significant progress in this direction was made during FY 1995. In collaboration with Dennis Burton (Scripps Research Institute) simian antibody fragments (Fabs) directed against the SIV envelope glycoprotein were cloned from a combinatorial filamentous phage display library constructed from the RNA of lymphocytes of a long-

term surviving SIV-infected monkey. These SIV Fabs exhibited different antigenic specificities and breadth of cross-reactivity. Significantly, one of the Fabs was shown to neutralize the infectivity of SIV in cell culture.

HONORS AND AWARDS

Robert M. Chanock, M.D.

Co-organizer of Symposium on *Modern Approaches to New Vaccines Including Prevention of AIDS*, Cold Spring Harbor Laboratory, Long Island, New York, October, 1994.

Invited Lecturer and Session Chairman, *Sixth Frank and Bobbie Fenner Conference in Medical Research*, Australian National University, Canberra, Australia, November 28-29, 1994.

Recipient of the Second Albert Sabin Vaccine Foundation Gold Medal and Invited Lecturer for *Second Annual Albert B. Sabin Lecture*, Cold Spring Harbor Laboratory, Long Island, NY, September 14, 1995.

Member of *National Academy of Sciences Committee on International Security and Arms Control, Working Group on Biological Weapons Control*, 1994 - 1995.

Vanessa M. Hirsch, D.V.M., D.Sci.

Permanent member, *NIH Study Section on AIDS and Related Research*, October, 1994 to 1997.

Scientific advisor, University of Oregon Regional Primate Research Center, 1995 to 1998.

Invited speaker at the *Annual Laboratory of Tumor Biology Meeting*, August, 1994.

Invited speaker at the *National Cooperative Vaccine Development Group (NCVDG) Meeting*, Reston, VA, September, 1994.

Chairperson and invited speaker, *12th Annual Symposium on Nonhuman Primate Models for AIDS*, Boston, MA, October, 1994.

Invited Speaker at the *Keystone Symposium on HIV Pathogenesis*, Keystone, CO, April, 1995.

Albert Z. Kapikian, M.D.

Invited to co-chair workshop on "Advances in Viral Gastroenteritis" at the *Seventh International Conference of Comparative and Applied Virology*, Montreal, Canada, October 12-16, 1994.

Invited to make a presentation at workshop at the *Seventh International Conference of Comparative and Applied Virology*, Montreal, Canada, October 12-16, 1994.

Invited to make a presentation at *NIAID Clinical Rounds*, December 2, 1994.

Invited to make a presentation at *Annual Meeting of the American Epidemiological Society*, March 23-24, 1995.

Elected as President-Elect of the *American Epidemiological Society*.

Invited to make presentation at the *Wyeth-Ayerst Rhesus Rotavirus Vaccine Investigators Meeting*, San Juan, Puerto Rico, March 31 - April 1, 1995.

Recipient of the Wyeth-Ayerst RRV Project Team Award, "In recognition of the ideas and scientific experiments that contributed to the development of the rotavirus vaccine for children" at the *Wyeth-Ayerst Rhesus Rotavirus Vaccine Investigators Meeting*, San Juan, Puerto Rico, March 31 - April 1, 1995.

Invited to make presentation at *Satellite Symposium on New Vaccines and Modern Vaccinology* during *International Conference in Commemoration of Louis Pasteur*, St. Petersburg, Russia, June 6-9, 1995.

Invited to give "Special Lecture" at *Sapporo International Symposium on Viral Gastroenteritis*, Sapporo, Japan, June 28-30, 1995.

Invited to make presentation at *Sapporo International Symposium on Viral Gastroenteritis*, Sapporo, Japan, June 28-30, 1995.

Invited to chair a session at *Sapporo International Symposium on Viral Gastroenteritis*, Sapporo, Japan, June 28-30, 1995.

Invited to make "Closing Remarks" (with Dr. Urasawa) at *Sapporo International Symposium on Viral Gastroenteritis*, Sapporo, Japan, June 28-30, 1995.

Invited to be member of "International Advisory Board" of Organizing Committee of *Sapporo International Symposium on Viral Gastroenteritis*, Sapporo, Japan, June 28-30, 1995.

Invited to make presentation to Tohoku Medical Association, Sendai, Japan, July 1, 1995.

Ching-Juh Lai, Ph.D.

Invited speaker and chairman, *Second International Meeting on Hepatitis C and Related Viruses*, San Diego, CA, July 31 - August 5, 1994.

Invited speaker, *WHO Workshop on Dengue and Japanese Encephalitis Virus*, San Diego, CA, August 6, 1994.

Invited speaker, *Fourth International Symposium on Positive Strand RNA Viruses*, Utrecht, The Netherlands, May 25-30, 1995.

Invited speaker, *Flavivirus Vaccine Research Session*, Utrecht, The Netherlands, May 25, 1995.

Invited speaker, *Fifth International Meeting for Society of Chinese Biomedical Association*, Vancouver, Canada, June 24-29, 1995.

Brian R. Murphy, M.D.

Member, Search Committee for Chief, Division of Viral Products, FDA, CBER.

Invited Speaker, *Australian Society for Microbiology*, presented the Bazely Oration entitled "Immunization Against Respiratory Viruses," Melbourne, Australia, September 26, 1994.

Chairman of Session on "Virology," at the Meeting *Molecular Approaches to the Control of Infectious Diseases*, Cold Spring Harbor, NY, October 5-9, 1994.

Scientific Advisory Board, Albert B. Sabin Vaccine Foundation, 1994.

Invited speaker, *The Eastern Pennsylvania Branch of the American Society for Microbiology Symposium on Vaccines: Preventive Strategies for the 21st Century*. Talk entitled: "The Development and Evaluation of Live Attenuated Respiratory Syncytial Virus Vaccines," Philadelphia, PA, December 8-9, 1994.

Invited speaker, *Keystone Symposia on Molecular Aspects of Viral Immunity*. Talk entitled "Progress Toward the Development of a Live Attenuated Respiratory Syncytial Virus (RSV) Vaccine," Keystone, CO, January 16-23, 1995.

Robert H. Purcell, M.D.

Invited speaker, *34th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Orlando, Florida, October 4-7, 1994.

Co-organizer and invited speaker, *The 25th International Symposium of the Princess Takamatsu Cancer Research Fund*, Tokyo, Japan, November 14-17, 1994.

Organizer and speaker (Annual Meeting) and Chairman, U.S. Delegation of the Hepatitis Panel, *U.S.-Japan Cooperative Medical Science Program*, Tokyo, Japan, January 22-24, 1995.

Invited speaker, *Symposium on Hepatitis A: Biology, Epidemiology and Prevention*, USUHS, Bethesda, MD, May 23, 1995.

Peter L. Collins, Ph.D.

Editorial Board, *Journal of Virology* and *Journal of General Virology*.

NIH Director's Award, 1995.

Invited member, *International Taxonomy Committee for Virology of the International Society of Microbiological Associations*, 1995.

James E. Crowe, Jr., M.D.

Invited speaker, *American Society for Virology 13th Annual Meeting*. Presentation entitled "Live attenuated subgroup A respiratory syncytial virus (RSV) vaccine candidates are highly attenuated in seronegative chimpanzees, yet immunogenic in chimpanzees, even in those with passively-acquired RSV antibodies," Madison, WI, July 9-13, 1994.

Invited to speak at the *1994 N.I.H. Research Festival*, session on "New Approaches to Vaccines for Respiratory Diseases." Presentation entitled "Immunoprophylaxis against disease caused by respiratory syncytial virus," Bethesda, MD, September 21, 1994.

Invited to speak at *Ebon Research Systems*, presentation entitled "The use of rodents in respiratory vaccine evaluation," October 27, 1994.

Invited to give *Pediatric Infectious Diseases Conference*, entitled "Current Approaches to Immunoprophylaxis Against Disease Caused by RSV," Johns Hopkins University Medical Center, Baltimore, MD, December 2, 1994.

Invited to give presentation at *Vaccine Symposium, Society for Pediatric Research*, entitled "Live attenuated mutants of respiratory syncytial virus correlate with level of attenuation for seronegative human infants," San Diego, CA, May 11, 1995.

Harold S. Ginsberg, M.D.

Recipient of *Bristol-Myers Squibb Fourth Annual Award for Distinguished Achievement in Infectious Disease Research*, December 7, 1994.

Honorary Doctor of Science Degree from Tulane University School of Medicine, June, 1995.

Suzanne U. Emerson, Ph.D.

Invited lecturer, Uniformed Services University of the Health Sciences, Animal Virology Graduate Course, January 25, 1995.

Public Health Service Honor Award, August 3, 1995.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00323-13 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Parainfluenza Virus Type 3 (PIV3) Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Tenured Scientist LID, NIAID

Others: Kenneth D. Dimock, Ph.D. N.I.H. Special Volunteer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00324-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory and Pre-Clinical Studies of Influenza Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: E. Kanta Subbarao, M.D. Senior Staff Fellow LID, NIAID
Eun Ju Park, Ph.D. Visiting Fellow LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

FDA (Epstein)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

2.0

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the past two years a new strategy was developed for the construction of live attenuated influenza A virus vaccine strains in which attenuating mutations are introduced into a cDNA copy of the PB2 gene by site-directed mutagenesis after which the mutant PB2 gene (in the form of full-length RNA transcripts of cDNA) is rescued into an infectious virus. This year we extended these initial findings by demonstrating the feasibility of introducing a putative *ts* mutation into the PB2 gene at amino acid (aa) residue 112, 265, 556, or 658 and found that each mutation indeed specified the *ts* and attenuation (att) phenotypes. Viable mutants were then constructed that had two or three *ts* mutations in the PB2 gene of the A/AA/6/60 virus. The AA mutant PB2 transfectants that possessed two or three PB2 *ts* mutations were highly attenuated and genetically stable in rodents and yet were able to induce protection against challenge with wild type virus. Sequential addition of single *ts* mutations into the PB2 gene brought about a step-wise increase in both temperature sensitivity and attenuation of the resulting transfectant virus. Such a PB2 gene bearing multiple mutations could be used alone, or in conjunction with another attenuating gene, to attenuate new epidemic influenza A viruses as they emerge in nature. The PB2 gene offers a distinct advantage over viral surface glycoprotein genes, such as the hemagglutinin or neuraminidase, as a site for an attenuating mutation(s) because it occupies an internal location in the virion and hence plays a minor role if any in inducing immunity to infection. This means that the PB2 gene (or other internal genes) can be transferred from an attenuated donor virus to any new influenza A virus by gene reassortment without compromising the immunogenicity of the new virus. Formulation and validation of this new strategy ushers in a new era in the development of live attenuated vaccines for influenza A virus. For the first time defined multiple attenuating mutations have been introduced by site-directed mutagenesis into a viral gene encoding an internal protein that can be readily transferred to new epidemic variants of the virus to achieve the desired optimal balance between attenuation and immunogenicity required of a safe and effective live influenza A virus vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00325-13 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: James E. Crowe, M.D. Clinical Associate (CO) LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID
Jinlin Du, M.D. Visiting Fellow LID, NIAID

COOPERATING UNITS (if any)

BIOQUAL, Inc., Rockville (Bradbury); Wyeth-Ayerst Research (Lubeck)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00326-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: E. Kanta Subbarao, M.D. Senior Staff Fellow LID, NIAID
 James E. Crowe, Jr., M.D. Research Associate LID, NIAID

COOPERATING UNITS (if any)

PRI DynCorp., Rockville, MD (Potash); Johns Hopkins University, Baltimore, MD (Clements); St. Louis University, St. Louis, MO (Belshe); Vanderbilt University, Nashville, TN (Wright); Wyeth-Ayerst Research, Radnor, PA (Zajac)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.25

PROFESSIONAL:

0.75

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Influenza A virus causes serious disease in infants less than six months of age, and thus, there is a need for a vaccine for this age group. 10^7 TCID₅₀ of a live attenuated cold-adapted influenza A virus vaccine given intranasally at two months of age together with the routine childhood immunizations and repeated at four or six months of age, induced a vigorous immune response in all vaccinees. Thus, it is possible to successfully immunize this young-age group with a cold adapted influenza A virus vaccine. A proposed strategy has been developed for use of live attenuated influenza alone or in conjunction with the licensed non-living vaccine in persons of all ages.

The bovine parainfluenza virus type 3 (BPIV3) vaccine, a live virus vaccine designed to protect against the antigenically related human PIV3, was found to be satisfactorily attenuated, stable genetically, poorly transmissible, and immunogenic in seronegative infants and young children older than six months of age. The response of twelve infants less than six months of age to 10^5 TCID₅₀ of virus was similar to that of the 27 older seronegative subjects demonstrating that this vaccine is safe and immunogenic in the young seronegative subject and that it continues to show promise as a vaccine candidate.

The live attenuated cold-passaged cp45 human PIV3 vaccine also was found to be satisfactorily attenuated, stable genetically, and immunogenic in seronegative infants and children when administered intranasally at a dose of 10^5 TCID₅₀.

A live attenuated intranasal bivalent subgroup A and B RSV vaccine is being developed. A candidate for the subgroup A component is the RSV cpts530/1009 mutant, a cold-passaged, temperature sensitive (cpts) virus. In preliminary Phase 1 studies this candidate vaccine at a dose of 10^4 pfu was found to be attenuated and genetically stable in seronegative infants and young children reproducing similar findings made in chimpanzees. A subgroup B vaccine candidate, designated RSV B1 cpts52/176 was attenuated for adults. Thus, significant progress has been made in the development of a RSV bivalent vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00327-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory and Pre-Clinical Studies of Parainfluenza Type 3 Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: Peter L. Collins, Ph.D. Tenured Microbiologist LID, NIAID

Scott Shors, Ph.D. IRTA LID, NIAID

Anna Durbin, M.D. Clinical Associate (CO) LID, NIAID

COOPERATING UNITS (if any)

Lederle-Praxis Biologicals (Tatem)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.25

PROFESSIONAL:

2.25

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Two live attenuated PIV3 candidate vaccines are in Phase 1-2 trials in human infants and children, namely the cold-passaged 45 mutant (*cp45*) of the JS wildtype human PIV3 and a bovine PIV3 that is antigenically related to human PIV3. The two vaccine candidates are safe, infectious, immunogenic, and genetically stable in seronegative infants and young children. The *cp45* virus replicates to very high titer in Vero cell culture grown on microcarriers. The Master Seed stock of the *cp45* virus maintains its temperature sensitive (*ts*), cold-adapted (*ca*), and attenuation (hamsters) phenotypes despite its high level of replication in the Vero microcarrier cultures. Thus, the large scale production of the *cp45* virus at this stage appears feasible.

A complete cDNA copy of the JS strain of human PIV3 has been constructed with the intent to rescue infectious virus from RNA transcribed from this synthetic cDNA copy of the RNA virus genome. This cDNA has the almost exact coding sequence of the wild type virus except for engineered mutations introduced by site-directed mutagenesis. These mutations were introduced to permit the virus rescued from the cDNA to escape neutralization by monoclonal antibodies that are used to neutralize the infectivity of the helper virus. In addition, mutations that mark the DNA have been introduced to permit unequivocal identification of rescued virus. The JS PIV3 cDNA has been modified for intracellular transcription of a full length vRNA sense genome for rescue into an infectious virus. Two systems of rescue of virus have been developed; a helper virus-based rescue system and a plasmid-based rescue system. Attempts to recover infectious virus from this full length cDNA using the two helper systems are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory and Pre-Clinical Studies of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: James E. Crowe, M.D. Clinical Associate (CO) LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID
Jinlin Du, M.D. Visiting Fellow LID, NIAID
Katalin Juhasz, Ph.D. Visiting Fellow LID, NIAID
Peter Collins, Ph.D. Tenured Microbiologist LID, NIAID

COOPERATING UNITS (if any)

BIOQUAL, Inc., Rockville (Bradbury); Wyeth-Ayerst Research (Lubeck); Scripps Research Institute (Burton); Intracell (Pilkington); PRI, Inc. (Potash)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.00

PROFESSIONAL:

2.00

OTHER:

3.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Significant progress was made in the development of a live attenuated RSV subgroup A vaccine by introducing additional attenuating mutations into mutants, such as *cp*RSV, that were not completely attenuated for fully susceptible humans. Several promising candidate RSV subgroup A vaccine candidates were produced and evaluated. The *cpts* 248/955, 248/404, and 530/1009 mutants derived from *cp*RSV were selected from a large panel of mutants for further study because they were attenuated, stable genetically, immunogenic, and able to induce protection in rodents or chimpanzees. Importantly, these mutants were able to induce a high level of resistance to wild type virus challenge even in chimpanzees passively infused with RSV antibodies at the time of immunization, a situation that simulates that of the young human infant whose serum usually contains passively acquired maternal RSV antibodies. The nucleotide sequence of the most promising attenuated viruses is being determined while that of the *cp*RSV parent mutant was completed this year. The *cpts*248/955 virus was found to be highly stable genetically in seronegative humans, but was not completely attenuated in these infants and young children. Importantly, the *cpts*530/1009 mutant appeared to be satisfactorily attenuated and stable genetically in the seven infected vaccinees evaluated thus far. The *cpts*530/1009 vaccine is our most promising candidate to date for the subgroup A component of a bivalent RSV vaccine.

Progress toward the development of the subgroup B component of the bivalent RSV vaccine was also made this year. It appears that the *cp*RSV B1/2B5 candidate vaccine virus sustained three independent mutations that contributed to its attenuation for cotton rats. The attenuation phenotype of this mutant was highly stable even after prolonged replication in immunosuppressed cotton rats. Derivatives of the *cp*RSV B1/2B5 mutant were obtained following mutagenesis and two further attenuated *ts* mutants were identified, namely *cp*RSV B1 176 and *cp*RSV B1 176/427. Both components of a bivalent subgroup A and B vaccine were able to replicate *in vivo* without apparent interference.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00368-13 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of the Genome of Respiratory Syncytial Virus (RSV)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Supervisory Microbiologist LID, NIAID

Others: Lili Kuo, Ph.D. Visiting Fellow LID, NIAID
Juan Cristina, Ph.D. Visiting Fellow LID, NIAID
Prabha Atreya, Ph.D. Visiting Fellow LID, NIAID
Rachel Fearn, Ph.D. Visiting Fellow LID, NIAID
Siba Samal, Ph.D. Special Volunteer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.75

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Previously, cDNAs were constructed to encode various truncated versions of the genome RNA of respiratory syncytial virus (RSV) in which the genome termini and putative transcription signals were retained but the viral genes were deleted and replaced with a reporter gene such as that for bacterial chloramphenicol acetyl transferase (CAT). When introduced into RSV-infected cells, the prototype RSV-CAT minigenome was replicated, transcribed and packaged into virus-like particles. This represents the first type of system in which genome-like RNAs of a nonsegmented negative strand RNA virus could be introduced into the viral replication cycle and rendered biologically active. Here, this system was used to identify and characterize the *cis*-acting replication and transcription signals in the RSV genome and to study the mechanism of gene expression. It also was adapted for the analysis of protein function and for the generation of infectious RSV from a cDNA of the complete genome (accompanying reports).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00372-13 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Roles of RSV Proteins in Host Immunity and Molecular Approaches to Vaccine Design

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Tenured Scientist LID, NIAID

Others: Brian R. Murphy, M.D. Head, RV Section LID, NIAID
Robert M. Chanock, M.D. Chief, LID LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.50

PROFESSIONAL:

0.50

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Infectious RSV was produced by the intracellular coexpression of five cDNAs encoding: (i) a complete version of RSV replicative intermediate RNA, and (ii) the four RSV proteins identified in other work as being necessary and sufficient to produce nucleocapsids competent for transcription and RNA replication (accompanying report). The ability to produce RSV from cDNA establishes the first available method for the direct engineering of infectious RSV.

One important use will be to facilitate and extend the characterization and development of a live attenuated RSV vaccine based on existing candidate vaccine strains. Specifically, the mutations responsible for desired phenotypic characteristics (attenuation, temperature-sensitivity, cold-adaptation, small plaque size, host range restriction, etc) of these existing viruses can now be directly identified and characterized by their individual insertion into the "wild type" genome. Desirable mutations from this menu can be mixed in various combinations to fine-tune phenotypes. This also will make it possible to modify vaccine virus to accommodate antigenic drift in circulating virus.

A second important use will be to explore new possibilities for improving live attenuated vaccine viruses. New types of attenuating mutations probably can be developed. The level of immunity associated with natural infection might be improved quantitatively or qualitatively in a recombinant virus by the inclusion of cytokine genes or additional T cell epitopes, by ablation of possible epitopes associated with reactogenicity, or by other manipulations. A third important use will be for exploring the roles of individual viral genes in virus replication and pathogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00498-09 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis, Processing and Functions of the Proteins of Human RSV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph. D. Tenured Scientist LID, NIAID

Others: Haim Grosfeld, Ph.D. Visiting Associate LID, NIAID
Prabha Atreya, Ph. D. Visiting Fellow LID, NIAID
Rachel Fearn, Ph.D. Visiting Fellow LID, NIAID
Siba Samal, Ph.D. Special Volunteer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.75

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We previously developed an experimental "rescue" system for respiratory syncytial virus (RSV) based on a cDNA-encoded "minigenome", called RSV-CAT RNA, bearing the foreign marker gene for chloramphenicol acetyl transferase (CAT). When synthesized *in vitro* and transfected into RSV-infected cells, RSV-CAT RNA was competent for RSV-specific transcription, replication and incorporation into virions (accompanying report). Here, we modified this system such that RSV as the source of complementing proteins was replaced by RSV proteins expressed from transfected cDNAs. Thus, the mix and relative amounts of complementing proteins could be varied. Three proteins, N, P and L, were necessary and sufficient for RNA replication (the synthesis of genome and antigenome [positive-sense replicative intermediate] RNAs). However, transcription (synthesis of subgenomic mRNA) by these three proteins alone yielded prematurely terminated mRNA. The coexpression of catalytic amounts of the M2 mRNA restored authentic transcription. Thus, the RSV "replicase" and "transcriptase" are not identical; the latter requires an additional elongation factor. At high concentrations of input M2 cDNA (yielding levels of protein comparable to those observed late during RSV infection), both transcription and RNA replication were inhibited. The M2 mRNA contains two overlapping orfs: the upstream orf encodes the M2 protein, and the second, internal orf lacks a known protein product. The effect of transcriptional elongation mapped to the former; the effect of inhibition of RNA synthesis to the latter. Coexpression (with N, P and L) of the NS1 protein resulted in inhibition of the synthesis of all RNAs, whereas coexpression of NS2 inhibited the synthesis of positive-sense RNAs. The M, SH, G and F proteins lacked detectable effect on RNA synthesis. Preliminary experiments indicated that transmissible particles were made when the mix of complementing proteins included the four envelope-associated proteins, M, SH, F and G, in addition to N, P, L and M2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00308-07 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vitro and *In Vivo* Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

Others: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00311-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Search For New Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

Others: Patrizia Farci, M.D. Visiting Scientist LID, NIAID
 Jens Bukh, M.D. Visiting Scientist LID, NIAID
 Norio Ogata, M.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

Hospital for Sick Children, Toronto, Canada (Phillips); Gene Labs, Inc., Redwood City, CA (Kim); Jefferson Medical College, Philadelphia, PA (Muñoz); National Institute of Virology, Pune, India (Arankalle); University of Maryland, Baltimore, MD (Strickland); King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (Ghabrah)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.58

PROFESSIONAL:

1.45

OTHER:

0.13

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1989 a possible paramyxovirus etiology for giant cell hepatitis was proposed, based on electron micrographic changes seen in the livers of patients with this disease. Attempts to transmit the disease to primates have been initiated.

Patients with thalassemia in Sardinia, Italy, receive monthly blood transfusions as therapy. Many of these patients have developed chronic hepatitis of unknown etiology. These patients are being studied for evidence of previously unrecognized hepatitis viruses. Similarly, clinical samples from patients with acute or chronic non-A, B, C, D, E hepatitis in the United States are being studied for biological, serological or molecular evidence of transmissible agents.

Patients with fulminant non-A, non-B hepatitis remain a diagnostic enigma and may be infected with one or more previously unrecognized viruses. We are attempting to transmit the disease to primates.

Evidence for the existence of an additional water-borne hepatitis virus has come from sero-epidemiologic studies in India and Saudi Arabia. From 50-100% of hepatitis cases in sixteen epidemics of water-borne hepatitis were caused by HEV but 1 water-borne epidemic was caused by neither HAV nor HEV. Similarly, in Saudi Arabia, 13.4% of acute hepatitis in adults could not be diagnosed as hepatitis A-E and appeared to be transmitted by nonparenteral means.

Some years ago, a hepatitis virus was reported to be transmissible from a non-A, non-B hepatitis patient to marmoset monkeys. The agent, called the GB agent, could be serially transmitted in marmosets and was partially characterized. Recently the GB agent was cloned and sequenced and shown to be distantly related to a previously unrecognized human virus. Others have independently discovered an additional human virus. The GB agent and the newly discovered human viruses are being studied in primates and *in vitro*.

The objectives of this project are to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A long-term objective is the development of passive and active immunoprophylaxis for these human pathogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00314-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Woodchuck Virus: Molecular Biological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Minshu Yu, Ph.D.	Senior Staff Fellow	LID, NIAID
	Atshushi Shimoda, M.D., D.M.S.	Visiting Scientist	LID, NIAID
	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

Division of Molecular Virology & Immunology, Georgetown University, Washington, DC (Gerin); New York State College of Veterinary Medicine (Tennant)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.58

PROFESSIONAL:

1.45

OTHER:

0.13

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient model for understanding HBV infections of humans. Several lines of research have been pursued. First, we found that the WHV X gene is very sensitive to alterations and is essential for viral replication in animal transfection experiments. Second, fine mapping of the X transcript promoter was used to identify the essential nucleotides in this cis-acting element. Third, we have extended our previous experiments on the characterization of the bi-directional promoter of WHV. Fourth, two WHV isolates were shown to develop surface antigenemia and liver tumors at significantly different rates (this last finding has important implications for understanding the molecular mechanisms involved in viral replication and in the oncogenic potential of hepadnaviruses). Fifth, an *in vitro* cell culture system for transfection of WHV was developed. Sixth, the role of the capsid protein in viral replication was studied. Seventh, the role of the envelope proteins in viral replication was examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00530-08 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Biological Studies of Hepatitis B Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Norio Ogata, M.D., D.M.S.	Visiting Scientist	LID, NIAID
	Atsushi Shimoda, M.D.	Visiting Scientist	LID, NIAID
	Fumihiko Sugata, M.D.	Visiting Scientist	LID, NIAID
	Raymond Tellier, M.D.	Special Volunteer	LID, NIAID
	Yu-Mei Wen, Ph.D.	Special Volunteer	LID, NIAID
	Minshu Yu, Ph.D.	Senior Staff Fellow	LID, NIAID
	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

Loeb Institute, Ottawa, Canada (Davis); Second Medical School, Shanghai, China (Xu); University of Massachusetts Medical Center, Worcester, MA (Robinson)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.42

PROFESSIONAL:

4.29

OTHER:

0.13

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued our characterization of a putative neutralization escape mutant of HBV that emerged in individuals who were vaccinated against hepatitis B. Two licensed vaccines were investigated for their ability to protect chimpanzees against challenge with the S gene mutant. Both recombinant vaccines provided protection against challenge with the mutant virus, suggesting that properly vaccinated individuals are not at risk of infection by the S gene mutant virus.

A bi-directional promoter complex was identified within the X gene of HBV, further emphasizing the complex genetic organization of this virus. We have recovered HBV sequences from the blood and liver of patients with chronic HBV infection and liver cancer. These sequences have been compared to determine if there are differences between the core genes circulating within hepatitis B virions and those that are integrated into liver cancer cells. Special emphasis was placed on core gene sequences, since their possible role in hepatocarcinogenesis is controversial. The sequence of HBV strains recovered from patients with fulminant hepatitis B is being determined. They will be compared with published sequences of HBV strains recovered from fulminant cases, since the relative importance of viral and host factors in fulminant hepatitis B has not been established. Hepatitis B virus is being used as a model system for evaluating the new naked nucleic acid approach to vaccine development. Results obtained with various recombinant vectors and immunization protocols are being compared with each other and with extensive experience with other approaches to hepatitis B vaccine development that have been studied previously in the laboratory. Strains of HBV of particular interest (such as from cases of fulminant hepatitis) are being subjected to a new PCR amplification procedure that amplifies virtually the entire genome for recovery of infectious cDNA. The amplified genomes will be sequenced and, possibly, biologically amplified and characterized in chimpanzees. Long term follow up of the NIAID's plasma-derived vaccine, developed in 1975 and the first successful subunit vaccine, has continued. The vaccine continues to be highly efficacious five years after administration to infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00569-06 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of Genetic Markers of Virulence and Adaptation to Cell-Culture of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Suzanne U. Emerson, Ph.D. Microbiologist LID, NIAID

Others: Ann Funkhouser, M.D. Research Associate (CO) LID, NIAID
Robert H. Purcell, M.D. Head, HV Section LID, NIAID
Gopa Raychaudhuri, Ph.D. IRTA Fellow LID, NIAID
Tina Schultheiss, Ph.D. Special Volunteer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.25

PROFESSIONAL:

3.46

OTHER:

0.79

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis A virus (HAV) is a picornavirus with a single-stranded RNA genome of approximately 7500 nucleotides. The wild-type strain of HAV grows poorly in cell-culture, generally is not cytopathic, and virus yields are low. A cell-culture adapted mutant has been selected which grows significantly more efficiently in cell-culture and which is attenuated for marmosets and chimpanzees. The objectives of this project are to determine the genetic basis for virulence and adaptation to cell culture of HAV in order to develop a strain of HAV suitable for use as an attenuated vaccine. The following advances in our understanding of HAV were made.

In an effort to increase replicative capacity, chimeric viruses were constructed from two or more HAV strains including a virulent human strain, an attenuated strain, a vaccine strain, a cytopathic strain, and a simian strain. The P2 region from a cytopathic strain of HAV was shown to confer the large focus phenotype but not the lytic phenotype of the cytopathic virus. Both halves of the P2 region were involved but not all of the mutations were required. HAV/7 chimeras containing the 2C gene of the simian virus grew less efficiently than did HAV/7 but intragenic 2C chimeras grew at an intermediate level. The first phenotype linked to the 2A gene was identified when we found that an engineered point mutation in 2A caused accumulation of viral capsids in the nucleus.

Chimeric viruses were constructed and assayed for the purpose of defining virulence genes. We identified the 2A gene as a second major HAV/7 determinant of attenuation for marmosets and obtained evidence that mutations in the 2A and 2C genes are almost totally responsible for the attenuation of the virus. The genetic determinants of virulence could be conferred through the 2C gene of a simian virus. Engineered mutations in the 2A gene decreased signs of histopathology but did not lower serum liver enzyme levels. Large deletions in the 5' noncoding region did not affect virulence but 5' noncoding mutations required for growth in MRC-5 cells resulted in lowered serum liver enzyme levels in combination with significant histopathology and high levels of virus excretion. A full length infectious cDNA of the attenuated HAV MRC-5 cell-adapted vaccine strain was constructed to serve as a genetic repository for the vaccine strain and to permit detailed molecular analysis of the virus it encodes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00570-06 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Hepatitis C Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

Others: Patrizia Farci, M.D. Visiting Scientist LID, NIAID
 Jens Bukh, M.D. Visiting Associate LID, NIAID
 Raymond Tellier, M.D. Special Volunteer LID, NIAID
 Masayuki Yanagi, M.D. Visiting LID, NIAID
 Roger H. Miller, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

Tokyo University, Tokyo, Japan (Shimizu); SmithKline Beecham, Rixensart, Belgium (Cabazon)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.75

PROFESSIONAL:

2.62

OTHER:

0.13

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis C virus (HCV) appears to be the major current etiological agent of transfusion-related non-A, non-B hepatitis. The HCV genome is a linear, positive-stranded RNA molecule of approximately 9,500 nucleotides and encodes a polyprotein of about 3,000 amino acids. Several stretches of amino acids in the HCV polyprotein share significant similarity with flavivirus and pestivirus proteins. Therefore, HCV is considered to be distantly related to these virus groups. The goal of this project is to increase our understanding of the molecular biology of this important human pathogen.

Extensive sequence analysis has been performed on the 5'NC, core, envelope 1 and hypervariable region (HVR1) of envelope 2 genes of over forty strains of HCV. In addition, neutralization assays, based upon blocking of attachment of virus to susceptible cells *in vitro* and prevention of infection of chimpanzees *in vivo*, have been developed and are being characterized for specificity and sensitivity. Prototype strains of the various genotypes of HCV, including some of those discovered in this laboratory, are being biologically amplified in chimpanzees and further characterized. Pools of virus-containing plasma will be packaged and distributed for further characterization, and used as challenge inocula in studies of passive and active immunoprophylaxis, etc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00596-05 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
In Vivo and In Vitro Studies of Hepatitis E Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Suzanne U. Emerson, Ph.D.

Microbiologist

LID, NIAID

Others: Sergei Tsarev, Ph.D., D.Sci.
Theo Heller, M.D.
Robert H. Purcell, M.D.

Staff Fellow
Research Associate
Head, HV Section

LID, NIAID
LID, NIAID
LID, NIAID

COOPERATING UNITS (if any)

University of Maryland School of Medicine (Strickland); WRAIR (Innis)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.92

PROFESSIONAL:

2.46

OTHER:

0.46

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Epidemics of enterically transmitted non-A, non-B hepatitis (hepatitis E) have been reported in Asia, Africa, and North America. Similar cases of sporadic hepatitis, presumed to be hepatitis E, account for up to 90% of reported hepatitis in countries where hepatitis E is endemic. Hepatitis E virus (HEV) has been implicated in fulminant hepatitis of pregnancy. This disease has a 20% fatality rate. That a viral agent was responsible for hepatitis E epidemics was first shown in 1983. On the basis of electron microscopy and molecular characterization it was proposed that HEV belongs to the calicivirus family. The goal of this project is to define the newly identified hepatitis E virus (HEV), determine the extent and pattern of its involvement in enterically transmitted hepatitis, and to develop a vaccine which prevents hepatitis E. We performed a seroepidemiologic study which showed that HEV is a major cause of acute viral hepatitis in Saudi Arabia but that the cases are mainly in expatriates and not in Saudis. We found that pregnant rhesus monkeys did not develop more severe disease than nonpregnant monkeys. Rhesus mothers infected with HEV did not transmit the virus to their fetus *in utero*. The ORF-3 protein was tentatively identified as an RNA binding protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00728-01 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Long Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Suzanne U. Emerson, Ph.D.

Microbiologist

LID, NIAID

Others:

Robert H. Purcell, M.D.
Raymond Tellier, M.D.
Jens Bukh, M.D.

Head, HV Section
Special Volunteer
Staff Fellow

LID, NIAID
LID, NIAID
LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.25

PROFESSIONAL:

1.12

OTHER:

0.13

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
- ☐ (a1) Minors
- ☐ (a2) Interviews

☒ (c) Neither

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The polymerase chain reaction has been adapted from Barnes' protocol for long PCR to incorporate a high level of an exonuclease-free, N-terminal deletion mutant of Taq DNA polymerase in combination with a very low level of a thermostable DNA polymerase exhibiting a 3' exonuclease activity. The reaction was modified to include a reverse-transcription step and was optimized for use with hepatitis C virus genomes as template.

It has been possible to amplify large amounts of 4 to 4.8 kb of hepatitis C virus genome from clinical samples. This amplified cDNA will be sequenced to complete genotyping studies of the virus, and used for the construction of full-length, possibly infectious, cDNA molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00500-06 LID

1994 to September 30, 1995

CT (80 characters or less. Title must fit on one line between the borders.)

and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1

ESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ching-Juh Lai, Ph.D.

Head, MVB Section

LID, NIAID

Barry Falgout, Ph.D.
Lewis Markoff, M.D.

Senior Staff Fellow
Medical Officer

LID, NIAID

LID, NIAID

NG UNITS (if any)

CH
atory of Infectious Diseases

ular Viral Biology Section

IE AND LOCATION

D, NIH, Bethesda, MD 20892

STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

APPROPRIATE BOX(ES)

a) Human subjects

☐ (a1) Minors

☐ (a2) Interviews

ARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

☐ (b) Human tissues

☒ (c) Neither

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00531-05 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analysis of Dengue Nonstructural Proteins NS2B and NS3

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D.

Head, MVB Section

LID, NIAID

Others: Barry Falgout, Ph.D.

Senior Staff Fellow

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00571-03 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amino Acid Substitution at the NS1-NS2A Cleavage Junction of Dengue Virus Polyprotein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michèle Pethel Microbiologist LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00572-03 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Dengue Virus Polyprotein NS3-NS4A-NS4B-NS5

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Barry Falgout, Ph.D. Senior Staff Fellow LID, NIAID
Annie Cahour, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00598-02 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dengue Type 4 Virus Mutants Restricted in Polyprotein Processing

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Hiroshi Kawano, M.D.

Visiting Associate

LID, NIAID

Others: Ching-Juh Lai, Ph.D.

Head, MVB Section

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00600-05 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering Viable Dengue Virus 5' and 3' Noncoding Region Deletion Mutants for Use in Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ruhe Men, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Alexander Pletnev, Ph.D.	Visiting Scientist	LID, NIAID
	Masayuki Tadano, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS (if any)

Walter Reed Army Institute of Research, Washington, DC (Hoke and Eckels)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.35

PROFESSIONAL:

2.10

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Success in cloning a full-length DEN4 cDNA which can be used to produce infectious RNA transcripts has created opportunities for detailed molecular analysis of the RNA genome and for development of new dengue vaccine strategies. A panel of 3' noncoding (NC) deletion mutants was previously recovered from RNA-transfected simian LLC-MK₂ cells. Most 3'NC deletion mutants exhibited restriction of growth and plaque formation in simian LLC-MK₂ cells. Interestingly, mutant 3'd 303-183 produced small plaques on mosquito C6/36 cells but grew in simian LLC-MK₂ cells to a high titer similar to that of wild type virus. To extend our vaccine strategy, mutant 3'd 303-183 and four other moderately restricted 3'd mutants were selected for evaluation of their infectivity and immunogenicity in rhesus monkeys. Mutant 3'd 303-183 induced a high level antibody response similar to that of the wild type virus, whereas other mutants induced low to moderate levels of antibodies as measured by radio-immunoprecipitation and virus neutralization. Unexpectedly, the results of a later preclinical trial showed that three 3' NC deletion mutants selected as candidate vaccines and the wild type DEN4 control, all prepared from cDNA and produced under GMP conditions failed to elicit an antibody response in monkeys. Experiments are in progress to determine if loss of immunogenicity of vaccine candidates could be explained by genetic alteration of DEN4 that might have occurred during recovery of dengue virus from mosquito cells transfected with RNA transcripts or during subsequent propagation of virus in simian FRhL cells that are approved for use in experimental vaccines destined for study in humans. In other studies, DEN4 cDNA was used to engineer deletions in the 5'NC region for functional analysis and for isolation of mutants that might show promise as candidate live vaccines. Several 5' NC deletion mutants that exhibited low to moderate efficiency for translation *in vitro* produced small plaques and exhibited reduced growth in cell culture. Among mutant constructs tested, deletion of nt 82-87 in the 5'NC region most severely reduced translation efficiency. Nevertheless, an infectious virus was recovered from simian LLC-MK₂ cells transfected with the RNA transcripts of mutant 5'd 82-87. The progeny virus produced small plaques on simian LLC-MK₂ cells and grew to low titer in these cells. Unlike wild type DEN4 or other 5' NC deletion mutants, mutant 5'd 82-87 failed to produce plaques on mosquito C6/36 cells and was also replication-defective in *A. aegypti* or *A. albopictus* following intrathoracic inoculation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00637-04 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of New Approaches to Vaccines Against the Tick-Borne Encephalitis Virus Complex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alexander Pletnev, Ph.D., D.Sci. Visiting Scientist LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID
 Michael Bray, M.D. Senior Staff Fellow LID, NIAID
 Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

USAMRIID (Huggins)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously, viable chimeric flaviviruses were constructed that contained tick-borne encephalitis virus (TBEV) structural protein CME or ME genes with the remaining genes derived from dengue type 4 virus (DEN4). The ME chimera retained the neurovirulence for mice of its TBEV parent from which its M and E genes were derived, but it lacked the peripheral invasiveness of TBEV. The ME chimera was subjected to mutational analysis in an attempt to reduce or ablate neurovirulence manifest when virus was inoculated directly into the brain. Three distinct mutations were independently associated with marked reduction of mouse neurovirulence. These mutations ablated: (i) the TBEV PreM cleavage site which is required for proper processing of M protein; (ii) the TBEV E (envelope glycoprotein) glycosylation site; or (iii) the first DEN4 NS1 (non-structural protein one) glycosylation site. Each of the three attenuated mutants was restricted in growth in both simian and mosquito cells. Significantly, parenteral inoculation of these attenuated mutants induced complete resistance in mice to fatal encephalitis caused by subsequent challenge with the highly neurovirulent ME chimera. These observations suggest a new strategy for developing a live attenuated TBEV vaccine.

Unlike the highly virulent TBEV, the wild type Langat virus (TP21 strain), the least virulent of all TBEV-complex flaviviruses, has low encephalitogenic potential and peripheral virulence and has not been reported to be associated with any human disease. In an attempt to identify the molecular basis for attenuation of TBEV-complex viruses, the sequence of the genome of wild type virus (TP21 strain) and a more attenuated strain of LGT derived from it (strain ES) were determined. Among the tick-borne flaviviruses, the LGT genome differs in length of its 3' noncoding region compared to TBEV or Powassan virus (a TBE virus of North America) genome. Analysis of the TP21 and E5 genome sequences revealed six amino acid differences in the polyprotein, one of them is a substitution Asn > Asp in position 387 of E protein, which probably is responsible for attenuation of E5 virus. Attenuating mutations which were identified by genetic analysis of the LGT strains will be introduced into chimeric LGT/DEN4 and TBEV/DEN4 genomes and progeny viruses will be analyzed for immunogenicity and loss of virulence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00681-03 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of Genetic Loci of Dengue Virus Neurovirulence in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Weiran Chen, Ph.D. Visiting Fellow LID, NIAID
 Michael Bray, M.D. Senior Staff Fellow LID, NIAID
 Kazufumi Hiramatsu, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

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SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

2.00

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Shortly after the first strains of dengue virus (DEN) were recovered fifty years ago, serial passage of DEN serotype 1 or 2 in mouse brain rapidly selected for neurovirulent mutants that concomitantly exhibited significant attenuation for humans. This rapid coordinate acquisition of mouse neurovirulence and attenuation for humans suggested that the genetic basis of these two phenotypes might be related. We had previously employed full-length DEN4 cDNA to construct a viable intertypic dengue type 1 or type 2 chimera that contained the C-PreM-E or only the PreM-E genes of DEN1 or DEN2 substituting for the corresponding genes of DEN4. Studies of the mouse neurovirulent mutant of the DEN2 NGC strain identified Glu₄₀₆ to Lys substitution in the envelope glycoprotein (E) as responsible for the acquisition of dengue type 2 virus mouse neurovirulence. This observation suggested that it might be possible to create an attenuating mutation in dengue viruses of all four serotypes as part of a general strategy for vaccine development. In an attempt to evaluate this strategy, we constructed DEN3/DEN4 chimeras that contained DEN3 C-PreM-E genes and expressed DEN3 antigenic specificity. A full-length DNA template of DEN3/DEN4 was prepared by *in vitro* ligation and used for transcription. Progeny virus recovered from RNA transfected mosquito C6/36 cells exhibited DEN3 antigenic specificity as determined by reaction with monoclonal antibodies. Gel electrophoresis of virus-infected cell lysates yielded the predicted viral protein pattern; *i.e.*, DEN3 C, PreM and E and DEN4 nonstructural proteins. Two amino acid substitutions, Thr₄₃₅Leu and Glu₄₀₆Lys analogous to mutations that, respectively, confer mouse neurovirulence on DEN4 or DEN2, were introduced into DEN3 E. Mutant chimera containing Thr₄₃₅Leu substitution, which ablates the potential glycosylation site sequence, produced E identical in size to wild type DEN3 E, indicating that the glycosylation site is normally not used. The Thr₄₃₅Leu mutant was not neurovirulent. In contrast, intracerebral inoculation of sucking mice revealed that the mutant chimera containing the Glu₄₀₆Lys substitution was neurovirulent, whereas its chimeric parent or parental DEN3 was not.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00682-03 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Intertypic Chimeric Dengue Viruses as Candidate Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael Bray, M.D. Senior Staff Fellow LID, NIAID

Others: Ruhe Men, Ph.D. Senior Staff Fellow LID, NIAID
 Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (If any)

LAB/BRANCH

Laboratory of Infectious Diseases

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.15

PROFESSIONAL:

0.90

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Dengue epidemics caused by the four dengue virus serotypes continue to pose a major public health problem in most tropic and subtropic regions. A safe and effective vaccine against dengue is still not available. The current strategy for dengue immunization favors the use of a vaccine containing all four serotypes. We previously employed full-length dengue type 4 virus (DEN4) cDNA to construct a viable intertypic dengue virus of type 1 or type 2 antigenic specificity that contained the capsid-premembrane-envelope (C-PreM-E) or only the PreM-E genes of DEN1 or DEN2 substituting for the corresponding DEN4 genes. Chimeras DEN1/DEN4 and DEN2/DEN4 which express the nonstructural proteins of DEN4 and structural proteins of DEN1 or DEN2, and therefore the antigenicity of type 1 or type 2, were used to infect rhesus monkeys. Control groups were inoculated with parental DEN1, DEN2, or cDNA-derived DEN4. Three of four monkeys immunized with DEN1/DEN4 developed neutralizing antibodies against DEN1, and were protected against DEN1 challenge. All four monkeys immunized with DEN2/DEN4 developed antibodies against DEN2 and were protected against DEN2 challenge. DEN1- and DEN2- immunized monkeys were protected against homologous challenge, but DEN4-immunized animals became viremic following cross-challenge with DEN1 or DEN2. In a second experiment, eight monkeys were immunized with an equal mixture of DEN1/DEN4 and DEN2/DEN4. Each animal developed neutralizing antibodies against both DEN1 and DEN2, and was protected against DEN1 or DEN2 challenge. Chimeric dengue viruses such as those evaluated in this study could be used to provide their serotype-specific antigens in a tetravalent human vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00683-02 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Determinants of Dengue Virus Mouse Neurovirulence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Kazufumi Hiramatsu, M.D. Visiting Associate LID, NIAID
Weiran Chen, Ph.D. Visiting Fellow LID, NIAID

COOPERATING UNITS (if any)

University of Hawaii, Honolulu, HA (Rosen)

LAB/BRANCH

Laboratory of Infectious Diseases

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Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00706-02 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Loci Responsible for Growth Restriction of Mouse-Adapted Dengue Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Ruhe Men, M.D. Visiting Scientist LID, NIAID
 Kazufumi Hiramatsu, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.25

PROFESSIONAL:

1.00

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Serial intracerebral passage of dengue type 1 or type 2 virus in mice was shown previously to select for mouse neurovirulent mutants which were attenuated for humans. Later, in a similar manner a neurovirulent mutant of DEN4 (strain H241) was selected by serial intracerebral passage in mice. In addition, DEN4 H241 neurovirulent (N) replicated less efficiently than DEN4 H241 parent (P) in simian LLC-MK₂ cells. An intratypic DEN4 chimera containing the C-PreM-E structural protein genes from DEN4 H241N also exhibited marked restriction of growth in LLC-MK₂ cells. Analysis of viral proteins produced in LLC-MK₂ cells by DEN4 H241N or its derived C-PreM-E chimera indicated that very little PreM was produced and that which was detected migrated slightly slower than the PreM of DEN4 H241P or its chimeric derivative. Recent evidence indicates that immature PreM-containing flavivirus virions are less infectious than the mature M-containing virus. Studies were performed to determine whether altered PreM or mutations in C or E might affect the normal processing of PreM that yields M which is normally the predominant product in the mature virion. Protein analysis indicated that DEN4 E, PreM, M and C were detected in a virion preparation of DEN4 H241P or its derived chimera. On the other hand, a virion preparation of DEN4 H241N or its derived chimera contained E, PreM and C, but not M. This suggested that cleavage of PreM to M was defective for DEN4 H241N and the genetic loci for the defect mapped within the C-PreM-E genes. There are six amino acid differences in the structural protein gene region between DEN4 H241P and DEN4 H241N: one in C, two in PreM and three in E. To identify mutations responsible for defective PreM cleavage, eight mutants were constructed from the intratypic DEN4 P chimeric virus that contained one or more amino acid substitutions that are present in the mutant C, PreM or E. Only mutant DEN4(H241P, S456), into which all three amino acid substitutions present in the E of DEN4 N were introduced, exhibited the PreM cleavage defect. Interestingly, chimeric mutants which contained both mutations in PreM processed PreM normally. This suggests that PreM interacts with E during virus maturation and changes in the latter can have a profound effect on processing of the former protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00333-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Longitudinal and Cross-Sectional Studies of Viral Gastroenteritis in Infants and Young Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

Others: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID
Mariam W. Watson, B.S. Biologist LID, NIAID

COOPERATING UNITS (if any)

Children's Hospital National Medical Center, Washington, DC (Kim)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.1

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although rotaviruses are established as the single most important cause of severe diarrhea of infants and young children world-wide being associated with 30-50% of such illnesses, the etiologic agents of at least 30% of severe diarrhea remain to be elucidated. Two groups of viruses, the astroviruses and caliciviruses, are known to cause infection in pediatric populations but their role as etiologic agents of severe diarrheal illness of infants and young children remains controversial. The goal of this project is to place these viruses in perspective with regard to their relative contribution to various forms of acute gastroenteritis in infants and young children. The availability of stool and serum specimens from several large-scale pediatric studies should enable us to address these issues. Two major studies provide the focus of this project. One is a longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless but otherwise normal children, and the other a cross-sectional study (1974-1991) of children hospitalized with gastroenteritis at Children's Hospital National Medical Center, Washington, DC. Our goal in the Junior Village studies has been to investigate the natural history of calicivirus and astrovirus infections in a longitudinal setting, whereas the Children's Hospital study provides materials that should allow us to determine the importance of calicivirus and astrovirus as agents of severe gastroenteritis requiring admission to the hospital. There is evidence from several studies that the caliciviruses and astroviruses both cause infection in infants but, as yet, their importance as etiologic agents of severe gastroenteritis is not established. This type of information must be obtained before priorities for vaccine development can be formulated. An essential feature of this study is the development of suitable assays to enable the study of specimens obtained from the settings described above.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00339-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation and Serotypic Characterization of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yasutaka Hoshino, D.V.M.

Visiting Scientist

LID, NIAID

Others: Ronald Jones
Albert Z. Kapikian, M.D.

Biologist
Head, Epid. Section

LID, NIAID
LID, NIAID

COOPERATING UNITS (if any)

University of Nebraska (Duhamel); Food and Drug Administration (Li); Instituto Adolfo Lutz, Brazil (Timenetsky)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.3

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

In this project we determined the major antigenic relationships of various rotavirus strains derived from humans and animals. Elucidation of the neutralization specificities of rotavirus is important in order to achieve a more comprehensive understanding of rotavirus epidemiology and for formulation of an effective strategy for vaccination. For example: (i) the bovine rotavirus strain NCDV-Cody which was originally isolated from a diarrheic calf and traditionally considered as the virulent counterpart for the attenuated NCDV-Lincoln (VP7 serotype 6) was shown to carry VP7 serotype 8 and VP4 serotype 6; (ii) the human rotavirus strain 69M (VP7 serotype 8) which was originally isolated from a child with diarrhea in Indonesia, was found to possess a new VP4 neutralization specificity which was closely related antigenically to equine rotavirus H-2 VP4; (iii) the human rotavirus strain MT19 which was originally isolated from a diarrheic child in Brazil was found to have a strong two-way cross-neutralization relationship with porcine rotavirus serotype 5 (OSU strain) and serotype 11 (YM strain); (iv) the vervet monkey rotavirus strain SA11 was shown to possess a distinct VP4 neutralization specificity which was related in a one-way fashion to rhesus monkey rotavirus MMU18006 VP4.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00340-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Studies of Rotavirus Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yasutaka Hoshino, D.V.M.

Visiting Scientist

LID, NIAID

Others:

Ronald Jones

Biologist

LID, NIAID

Albert Z. Kapikian, M.D.

Head, Epid. Section

LID, NIAID

COOPERATING UNITS (if any)

Ohio State University (OARDC), Wooster, Ohio (Saif); University of Nebraska, Lincoln, Nebraska (Duhamel)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.8

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Previously, in studies involving a semi-homologous system of gnotobiotic newborn pigs and a virulent porcine rotavirus strain (SB-1A) and an avirulent human rotavirus strain (DS-1) and their reassortants, we demonstrated that: (i) the third (VP3), fourth (VP4), ninth (VP7), or tenth (NS28) porcine rotavirus gene each play an important independent role in the virulence of rotavirus infection in piglets; and (ii) all four of the porcine rotavirus virulence-associated genes are required for the induction of diarrhea and the shedding of a virus by piglets. These observations suggested a potential new strategy for attenuation of wild-type human rotaviruses of major epidemiologic importance and its application to the development of a safe and effective vaccine.

Based on this strategy efforts were made to generate four human x bovine rotavirus reassortants, each of which has: (i) the VP4-encoding gene from human rotavirus Wa (VP4:1A); (ii) the VP7-encoding gene from human rotavirus D (VP7:1), DS-1 (VP7:2), P (VP7:3), or ST3 (VP7:4); and (iii) the remaining nine genes including the VP3-encoding gene and NS28-encoding gene from bovine rotavirus UK. In addition, we successfully generated two human x bovine rotavirus reassortants, each of which had the VP4-encoding gene from human rotavirus Wa (VP4:1A) or DS-1 (VP4:1B) and the remaining ten genes from bovine rotavirus UK. More recently, we have generated two additional human x rhesus rotavirus reassortants, each of which has the VP4-encoding gene from human rotavirus Wa (VP4:1A) or DS-1 (VP4:1B), the VP7-encoding gene from human rotavirus DS-1 (VP7:2) and the remaining nine genes from rhesus rotavirus MMU18006. Such strains may be important in the development of an optimally effective rotavirus vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00341-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Live Attenuated Rotavirus Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

Others: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID
 Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

Institute of Biomedicine, Caracas, Venezuela (Pérez-Schael; Rojas); Univ. of Rochester (Treanor); Johns Hopkins University (Clements); Univ. of Tampere (Vesikari, Ruuska); Institute of Nutrition, Peru (Lanata); Wyeth-Ayerst Research; Secretech, Inc. (Schafer); Dyn-Corp-PRI (Potash); Vanderbilt University (Wright)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.8

PROFESSIONAL:

0.9

OTHER:

2.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses are the single most important etiological agents of severe diarrheal disease in infants and young children in both the developed and developing countries. These viruses are estimated to cause 30 to 50% of such illness in the US as well as world-wide. The consequences of such illness in developing countries are particularly grim because severe rotavirus disease in these regions is estimated to cause over 870,000 deaths each year. Thus, the need for an effective vaccine able to prevent severe rotavirus disease is clear.

We have taken a "Jennerian" approach to vaccine development. This involves the use of a live animal virus that is: (i) antigenically related to its human virus target, (ii) attenuated for humans, and (iii) able to induce protective immunity against the human virus. Initial studies were performed with a simian rotavirus, rhesus rotavirus (RRV), that is closely related to human rotavirus serotype 3. Impressive protective effect was observed against serotype 3 human rotavirus disease but efficacy was variable against other rotavirus serotypes. We, therefore, developed a modified "Jennerian" approach that involved formulation of a quadrivalent rotavirus vaccine containing: (i) rhesus rotavirus (RRV) (VP7 serotype 3), and (ii) three human rotavirus-RRV reassortants, each possessing ten RRV genes and a single human rotavirus gene that encodes VP7 (the major protective antigen) serotype 1, 2, or 4 specificity.

This has been a banner year because the results of two recently completed studies in the United States indicate that the quadrivalent formulation provided a high level of protective efficacy against severe rotavirus diarrheal disease. Under the auspices of our licensee, Wyeth-Ayerst Research, a multi-center (23 sites), three cell prospective, double-masked, placebo-controlled efficacy trial covering a period of two rotavirus seasons was performed. Protective efficacy against serious rotavirus disease was 82%. In addition there was a 78% reduction in need for medical visits for rotavirus disease. These encouraging observations were confirmed and expanded during a second large multi-center trial. Quadrivalent vaccine administered at a ten-fold higher dosage was 80% protective against severe rotavirus diarrheal disease. Most important was the observation that the quadrivalent vaccine was 100% effective in preventing dehydrating illness, the most severe form of rotavirus diarrhea.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00342-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although the use of the electron microscope (EM) by the Laboratory of Infectious Diseases (LID) reached its peak activity in the 1970's with the discovery of the Norwalk virus, Hawaii virus, Montgomery County virus, and hepatitis A virus, as well as the initial detection in the United States of human rotavirus (which was discovered in Australia), there has been a marked resurgence of usage of the EM recently. This has resulted from the expression of recombinant 27nm virus-like particles bearing the outer capsid antigens of Norwalk virus (by others) and Desert Shield, Hawaii, and Toronto viruses (by LID scientists)--all associated with acute gastroenteritis and all members of the family Caliciviridae. The utilization of the EM has proved to be essential because none of these fastidious viruses has yet been propagated successfully in cell culture. Therefore, the EM remains as: (i) the only method for the detection of the 27nm recombinant virus-like particles as well as the native 27nm virus particles, and (ii) the only method for the demonstration of specific antigenic relationships among these recombinants (by immune electron microscopy). In addition, the EM has been an important adjunct to many of the projects of the section providing seminal information in the progress of molecular biologic (as well as other) studies. The scope of the use of the EM is supported by the fact that approximately ninety individual experiments were conducted by electron microscopy since the previous annual report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00343-14 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

Baylor College of Medicine (Estes); U.S. Naval Medical Research Institute (Hyams, Sharp, Savarino);
 University of Tampere, Finland (Vesikari)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.3

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Norwalk virus and related 27nm caliciviruses are an important cause of epidemic viral gastroenteritis that occurs in family, school, group, institutional, or community-wide outbreaks affecting adults, school-aged children, family contacts, and some young children as well. Most information concerning the epidemiology of this group of viruses has been generated using diagnostic assays that employed either native Norwalk virus antigen present in or purified from human stools or, more recently, recombinant Norwalk VLPs to detect serologic responses. This year, large-scale epidemiologic studies were initiated using newly developed diagnostic assays that employed recombinant calicivirus capsid antigens that represent viruses serotypically distinct from Norwalk virus. The association of several gastroenteritis outbreaks with Hawaii virus or Toronto virus, each serotypically distinct, indicates that a better understanding of the epidemiology of caliciviruses will emerge from this approach.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00507-07 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of Rotavirus Serotypes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D.

Senior Staff Fellow

LID, NIAID

COOPERATING UNITS (if any)

University of Rochester, Rochester, NY (Madore & Dolin); University of Tampere, Tampere, Finland (Vesikari)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00533-06 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Rotavirus Proteins with Monoclonal Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

University of Tampere, Tampere, Finland (Vesikari)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00534-06 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Non-Group A Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio (Saif);
Capital Institute of Pediatrics, Beijing, China (Qian)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00573-04 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Rotavirus Proteins in *Salmonella* Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

University of Maryland, Baltimore, Maryland (Levine & Hone); Washington University (Curtiss);
University of Missouri (Parker)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00604-05 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cold-Adaptation of Human Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID
 Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

DynCorp (Potash)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The selection of cold-adapted mutants of selected human rotaviruses of major epidemiologic importance was reported last year. These mutants included strain D (VP4:1A; VP7:1), DS-1 (VP4:1B; VP7:2), Wa x DS-1 (VP4:1A; VP7:2), and Wa x P (VP4:1A; VP7:3). Selected mutant vaccines are currently under clinical evaluation. In an attempt to provide antigenic coverage for each of the four epidemiologically important VP7 serotypes (G1, G2, G3, and G4), we passaged a Wa x ST3 (VP4:1A ; VP7:4) reassortant serially in primary African green monkey kidney cells at progressively lower suboptimal temperature (30° C, 28°C, and 26°C). Triple plaque purification of each mutant selected after tenth serial passages is in progress at the temperature of that passage series.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00605-04 LID

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Infection of Chimpanzees by Human Rotavirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

Others: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID
Eileen N. Ostlund, D.V.M. IRTA Fellow LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

Georgetown University/Twinbrook (London); Bioqual, Inc., Rockville, MD (Bradbury)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

INACTIVE 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00641-04 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Neutralizing Epitopes on Rotavirus Outer Capsid Proteins (VP4 and VP7)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

Genetic Therapy (Gorziglia)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One serotype-specific and three serotype-cross-reactive anti-VP7 neutralizing monoclonal antibodies (N-mAbs) were utilized to study the neutralization epitopes involved in the formation of antigenic sites on porcine rotavirus Gottfried strain (serotype 4). Single, double, or triple neutralization-resistant mutants were selected in the presence of these N-mAbs *in vitro*. Sequence analysis of the gene encoding VP7 of such mutants revealed that: (i) the amino acid (aa) that is substituted at a specific position on the mutant VP7 selected by a single N-mAb can vary, resulting in variants which exhibit antigenic differences; (ii) in addition to variable regions VR-5 (aa 87-100), VR-7 (aa 141-150), VR-8 (aa 208-224), and VR-9 (aa 235-242), aa 290 and 291 in the constant region of the VP7 are involved in neutralization, and (iii) VR-5, VR-7, VR-8, VR-9, and aa 290 and 291 are functionally related to one another. In order to further analyze neutralization sites on the VP7 and to study possible synergistic or antagonistic effects among the N-mAbs employed in this study, antigenic variants were generated in the presence of three N-mAbs. Nucleotide sequence analysis of the VP7-encoding gene of the mutants failed to demonstrate synergistic or antagonistic effects among the mAbs used in the selection of neutralization escape mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00642-04 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of Norwalk and Norwalk-Like Viruses (27 nm Viruses)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID

Others: Judy F. Lew, M.D. Senior Research Invest. LID, NIAID
 Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID
 Stanislav Sosnovtsev, Ph.D. Visiting Fellow LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Norwalk and Norwalk-like human caliciviruses are important etiologic agents of epidemic nonbacterial gastroenteritis. A major research obstacle in the study of these viruses has been our inability to grow them in cell culture. The primary goal of this project is to characterize viral genetic determinants that are responsible for the antigenic diversity and epidemiologic importance of these human pathogens. Baculovirus-expressed self-assembled virus-like particles (VLPs) from the Desert Shield virus (DS395), Toronto virus (TV24), and Hawaii virus (HV) were used to develop diagnostic assays and serologic reagents to examine antigenic relationships among these and other calicivirus reference strains. Relationships established with these recombinant DNA-based assays should provide a framework for the development of a provisional human calicivirus serotypic classification system until neutralization assays are available. In addition, recombinant calicivirus proteins are being used to study basic biological features of the virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00684-02 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Serological Characterization of Equine Rotaviruse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eileen Ostlund, D.V.M., Ph.D. IRTA Fellow LID, NIAID

Others: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00685-02 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Studies of the Cold-Adapted Human Rotavirus Phenotype

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eileen Ostlund, D.V.M., Ph.D.

IRTA Fellow

LID, NIAID

Others: Yasutaka Hoshino, D.V.M.

Visiting Scientist

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1994 - 1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00707-02 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Genetic Determinants Required for the Replication of Caliciviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Y. Green, Ph.D.

Senior Staff Fellow

LID, NIAID

Others: Stanislav Sosnovtsev, Ph.D.

Visiting Fellow

LID, NIAID

COOPERATING UNITS (if any)

Uniformed Services University of the Health Sciences, Bethesda, MD (Monroe, Vincent)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Caliciviruses, represented by the prototype Norwalk virus, are the major cause of acute nonbacterial epidemic gastroenteritis in humans. Our continued inability to cultivate any of the etiologic human caliciviruses of human gastrointestinal disease in cell culture has been a major research obstacle. We have used a cultivatable feline calicivirus, URB, as a model for the study of calicivirus biology and replication. The sequence of the 7.7 Kb RNA genome of the URB strain was determined from cloned cDNA fragments and this information was utilized to construct a full-length cDNA copy of the genome downstream from the T7 RNA polymerase promoter. Full-length synthetic RNA transcripts prepared from this clone were infectious when transfected into feline kidney cells, thus providing us with an opportunity to gain important insights into the replication of this family of viruses and their relationship to other positive-strand RNA viruses. Experiments are in progress utilizing the infectious feline calicivirus cDNA clone (as a surrogate for Norwalk virus) to study receptor binding, infectivity of viral RNA, mapping of gene products in the viral genome, protein processing, viral pathogenesis, and the mechanisms responsible for host cell restriction in cell culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00729-01 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthetic Analogs of Rotavirus Genomic RNA Expressing a Foreign Marker Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: **An-Dao Yang, M.D.**

Visiting Associate

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The genome of rotavirus consists of eleven segments of double-stranded RNA (dsRNA) which encode six structured proteins (VP1, VP2, VP3, VP4, VP6, and VP7) and five nonstructural proteins (NS53, NS34, NS35, NS20, and NS26). Each genomic RNA segment contains a methylated cap 5' - sequence M⁷GpppG(m)GPy at 5' end. No polyadenylation tract is found at 3' end and RNA transcripts are not polyadenylated. The 5' and 3' end of each genome segment contain distinct, unrelated terminal consensus sequence of seven to ten nucleotides. They are assumed to be important *cis*-acting signal. The termini are also thought to contain sequences important in packaging and in the regulation of rotavirus gene expression at the level of transcription replication, and translation. However, the precise roles of the termini in the regulation of rotavirus gene expression are not known, little has been learned about the *cis*-acting signal from our previous work that indicated the 3'-terminal *cis*-acting signals required for replication are the nineteen 3'-terminal which include the 7-nt-terminal consensus sequence together with 12nt of adjoining, less-well-conserved sequence [Gorziglia and Collins, 1992].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00370-13 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis Studies of Simian Immunodeficiency Virus (SIV) Infection of Macaques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Vanessa Hirsch, D.V.M., D.Sc. Visiting Scientist LID, NIAID

Others: Simoy Goldstein, Ph.D. Visiting Scientist LID, NIAID
 Barbara Campbell, Ph.D. Staff Fellow LID, NIAID
 William R. Elkins, D.V.M. Senior Veterinarian LID, NIAID
 Harold Ginsberg, M.D. Expert LID, NIAID
 Raymond Langley, Ph.D. IRTA LID, NIAID

COOPERATING UNITS (if any)

The Henry Jackson Foundation (Lewis, Brown); Southwest Foundation for Biomedical Research (Allan); University of Nebraska (Stevenson); The Ohio State University (Johnson); Genelabs Inc. (Lifson); Beth Israel Hospital, Boston (Letvin)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Immunodeficiency Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

6.5

PROFESSIONAL:

3.5

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major goal of this project is the elucidation of SIV pathogenesis in experimentally- infected macaques, utilizing strains derived from sooty mangabeys (SIVsm) and African green monkeys (SIVagm). A SIVagm strain (SIVagm9063) which induces immunodeficiency in experimentally infected pig-tailed (PT) macaques was isolated. An infectious, molecular clone of this isolate also induced AIDS in PT macaques, whereas, inoculated rhesus (RH) macaques and AGM, the natural host, remained healthy. Significant differences in virus load were observed during primary infection of PT macaques, RH macaques and AGM by this cloned virus. A large burst of virus expression was observed during the primary infection of PT macaques, whereas virus levels were significantly lower in RH or AGM. These data suggest that species-specific virulence is associated with the extent of *in vivo* viral replication. With respect to SIVsm strains, a SIVsmE543 clone was derived from a highly pathogenic isolate of SIV from an immunodeficient RH macaque, E543. Like primary clinical isolates of HIV-1, this cloned virus appears to be resistant to neutralization *in vitro* by serum of monkeys that neutralized related laboratory strains of SIVsm with high efficiency. Two of four animals inoculated the SIVsmE543 cloned virus failed to seroconvert despite high viremia, and one of these animals was sacrificed with pneumonia at sixteen weeks. We have also pursued the study of SIV-infected macaques which were infected with either molecularly cloned SIVsm62d or uncloned SIVsmE660 but failed to develop AIDS. High primary viremia which persisted into the asymptomatic phase of infection was characteristic of animals that progressed to AIDS; low level of primary viremia with sustained control of virus level was observed in nonprogressors. Three of four SIVsmE660 nonprogressors had been immunized previously with a highly attenuated vaccinia virus (MVA)-SIV recombinant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00686-03 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vaccine Strategies for Control of Simian Immunodeficiency Virus (SIV) Infection of Macaques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Vanessa M. Hirsch, D.V.M., D.Sc. Visiting Scientist LID, NIAID

Others: Simoy Goldstein, PhD Visiting Scientist LID, NIAID
 Jaokim Glammann, Ph.D. Visiting Associate LID, NIAID
 William R. Elkins Senior Veterinarian LID, NIAID

COOPERATING UNITS (if any)

LVD, NIAID, NIH (Moss); Genelabs (Fuerst, Lifson); The Ohio State University (Johnson); The Henry Jackson Foundation (Zack, Lewis); Vanderbilt University (Montefiori); Bristol Myers Squibb (Haigwood); Chiron Corporation (Van Nest); Beth Israel Hospital, Boston (Letvin); University of Pennsylvania (Doms, Hoxie)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Immunodeficiency Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

2.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we used the SIV/macaque model of AIDS for the evaluation of potential vaccine strategies for control of human HIV disease. Emphasis was given to the evaluation of an attenuated vaccinia virus (modified vaccinia virus Ankara; MVA) recombinant expressing the SIVsmH4 gag-pol and env (MVA-SIV) which were compared to a similar recombinant constructed on a Wyeth strain vaccinia virus background (Wyeth-SIV). The Wyeth vaccine virus is a standard vaccine strain used previously in many smallpox immunization campaigns. Four rhesus macaques per immunogen were immunized four times over a 46-week period. Whereas, the MVA-SIV boosted immune response following sequential inoculations, the Wyeth-SIV recombinant only boosted at the second immunization and antibody levels declined subsequently. The antibody response was then boosted with psoralen-inactivated SIV administered without adjuvant. Although immunization did not prevent infection following intravenous challenge with uncloned SIVsm, the dynamics of virus replication were significantly different. Three of the MVA-SIV-vaccinees exhibited sustained control of viremia throughout one year following challenge. This pattern of markedly reduced viremia was associated with maintenance of normal lymphocyte subsets and disease-free status, analogous to longterm nonprogressors of HIV-1 infection. In contrast, three of the Wyeth-vaccinia SIV recombinant and three of the naive control group developed AIDS by one year. These data suggest that immunization with MVA-SIV significantly and beneficially modified the subsequent pathogenesis of SIV infection. As part of our interest in passive immunoprophylaxis and passive immunotherapy we generated a combinatorial phage library of the antibody repertoire of a seven-year, healthy survivor of SIV-infection. Initial screening of the phage-Fab library has yielded four distinct Fabs that bind specifically to SIV envelope. One of these Fabs neutralizes the infectivity of SIV *in vitro*. Passive transfer of IgG purified from the serum of a long-term surviving macaque to naive monkeys 24 hours after SIV challenge had a definite therapeutic effect that beneficially retarded progression of disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00730-01 LID

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Cotton Rat as a Small Animal Model for Human Immunodeficiency Virus (HIV-1)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Harold S. Ginsberg, M.D. Expert Scientist LID, NIAID

Others: Raymond Langley, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

Virion Systems, Inc. (Prince)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Immunodeficiency Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this work is to determine whether the cotton rat, which is highly susceptible to infection with many viruses, can be infected with the human immunodeficiency virus (HIV-1). Two inbred species of cotton rats, *Sigmodon hispidus* and *S. fulviventer*, were employed. The LAI laboratory-adapted strain of HIV-1 was used, and both species were infected as determined using PCR and antibody assays. The *S. fulviventer* strain of cotton rats, however, proved to be the more susceptible. Although virus could not be isolated in cultures of CEM human cells, it was possible to effect two serial transmissions of the virus to other cotton rats. Experiments have been recently initiated using a freshly isolated virus, and these animals were infected early and appear to be carrying significantly more virus in their PBMCs.

LABORATORY OF INTRACELLULAR PARASITES
Rocky Mountain Laboratories
Hamilton, Montana

1995 Annual Report
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Annual Report
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1994 to September 30, 1995

Summary

Scientists in the Laboratory of Intracellular Parasites (LICP) study infectious diseases caused by obligate and facultative intracellular bacterial pathogens. These include *Chlamydia*, *Mycobacteria*, *Rickettsiae*, and bacterial enteropathogens. Molecular, biologic, and immunologic approaches are used to delineate the structure/function relationship of parasites in their interaction with host cells and to delineate immune mechanisms that function in the development of acquired immunity to infection. Trafficking of biosynthetic precursors through cellular organelles to the chlamydial vesicle and modification of that vesicle by pathogen derived proteins are current areas of research. Molecular biology and modern chemistry are being used to elucidate the details of biosynthetic pathways leading to the synthesis of mycobacterial mycolic acids. The results of this work are being used to design new anti-mycobacterial drugs that can targeted the biosynthesis of mycolic acids. *Escherichia coli* heat-labile enterotoxin and cholera toxin are being investigated by site-directed mutagenesis and other biochemical techniques to identify structures that function in the toxic and mucosal immunomodulatory properties of these proteins. Animal models are being used to study acquired immunity to chlamydial infection of mucosal surfaces with specific emphasis on delineating the role of T-cells in protective immunity. The results of this work are being used to formulate rational strategies for the development of subunit and recombinant vaccines for the prevention of chlamydial caused sexually transmitted diseases of humans.

Research Highlights

Immunology Section: The Immunology Section is under the direction of Dr. Harlan Caldwell. There are two major research themes within the Immunology Section; (i) utilization of a murine model for the study of protective immune responses to chlamydial infection of the genital tract mucosa, and (ii) the development of a safe and efficacious vaccine for the prevention of chlamydial caused sexually transmitted diseases of humans. A murine model of chlamydial genital tract infection has been developed and is being used to study the basic mechanisms of protective immunity. Knock out (KO) mice and adoptive immunization using T subsets isolated from immune mice are being used to define the role T cells in acquired immunity to chlamydial infection. The results of this work show that MHC class II and CD4⁺ deficient strains of mice fail to resolve chlamydial genital tract infection whereas β_2 -microglobulin and μ Mt^{-/-} (B cell deficient)

mice resolve infection similarly to C57BL/6 parent control mice. It was also shown that splenic CD4⁺ T cells but not CD8⁺ T cells isolated from immune mice were capable of transferring significant levels of protective immunity to immunocompetent naive mice. These findings indicate that CD4⁺ T-cells are essential for the development of protective immunity to chlamydial genital tract infection and implicate Th1 cells as the major protective T cell subset. Studies have also been done to define the kinetics of natural antigen processing and presentation of chlamydial proteins by bone marrow derived macrophages to protective CD4⁺ T cells. This work has provided critical information for the isolation of naturally processed chlamydial peptides from MHC class II molecules. Future work will focus on the identification of chlamydial specific peptides isolated from class II molecules that are recognized by protective CD4⁺ Th1 cells and defining the mechanism(s) by which CD4⁺ Th1 cells inhibit chlamydial growth. This information is being used for the rational design of synthetic or recombinant vaccines that can be targeted to activate protective CD4⁺ Th1 immune responses against chlamydia at the genital tract mucosa.

Mycobacterial Section: The Mycobacterial Section is under the direction of Dr. Clifton Barry, III. The overall goals of the Section relate to understanding the structure and function of the mycobacterial outer cell wall complex. Within this framework, projects in the unit include; (i) characterization of the biosynthetic pathway for mycolic acids in *Mycobacterium tuberculosis* (MTB) and identification of roles for the various modifications of these cell wall lipids to survival of the organism within macrophages, (ii) characterization of the macromolecular structure of the mycobacterial outer cell wall and the relation of substructures to the overall permeability and drug-resistance of the organism, (iii) molecular approaches to understanding the mechanism of action of Isoniazid, a drug purported to have a direct effect on the biosynthesis and assembly of the cell wall complex, and (iv) characterization of the KatG and OxyR-dependent inducible genetic response to oxidative stress in mycobacteria.

Cyclopropanation of mycolic acids is limited to pathogenic strains of mycobacteria, including MTB. Section members have used heterologous expression in saprophytic strains of mycobacteria (*M. smegmatis*) to identify two separate genes whose protein products function to cyclopropanate mycolic acids in MTB. By coexpressing both of these genes MSMEG has been made to produce the major mycolic acid typical of MTB which contains two cis cyclopropanes. We have shown that cyclopropanation of mycolic acids is an important protective mechanism which facilitates the survival of pathogenic species to colonize macrophages in spite of a barrage of toxic oxygen species produced by these cells. Section members (in collaboration with Dr. Hiroshi Nikaido, UC Berkeley and Dr. Gurdayal Besra, CSU, Ft. Collins) have also been involved in showing that the outer cell wall complex is characterized by a state of exceptionally low fluidity as measured by differential scanning calorimetry (DSC). Cyclopropanation of mycolic acids structurally raises the phase transition temperature and so directly contributes to permeability of the cell wall. The mechanism of isoniazid action is being investigated using a combined genetic and biochemical approach to identifying the mutations which are clinically relevant

in conferring isoniazid resistance. These investigations involve examining lipid and protein synthetic profiles following isoniazid treatment and complementation of resistant strains with libraries from clinically resistant isolates. In work with the Section's CRADA partner, PathoGenesis Corp. (Seattle, WA), unit members have characterized the response of MTB to oxidative challenge *in vitro* and have shown that this response is not typical of other bacteria in that pathogenic mycobacteria have no functional OxyR response, while saprophytic mycobacteria do. Although there is not a typical OxyR response, there is an upregulation of one gene, KatG, in response to oxidative challenge. In addition, we have shown that the KatG regulation and resistance to hydrogen peroxide killing *in vitro* is an extremely growth phase dependent phenomenon. This has led us to characterize stationary phase MTB in some detail in an effort to understand this enhanced resistance and perhaps allow an analysis of bacterial persistence.

Host-Parasite Interactions Section: The Host-Parasites Interactions Section is under the direction of Dr. Ted Hackstadt. The major research interest within the Host-Parasite Interactions Section involves the cell biology of obligate intracellular parasites. *Chlamydia trachomatis* and *Coxiella burnetii* are bacterial obligate intracellular parasites that occupy distinct vacuolar niches within eucaryotic host cells. We have employed immunofluorescence, cytochemistry, fluorescent vital stains, and fluid phase markers to characterize the vacuolar environments of these pathogens. By all criteria applied, *C. burnetii* resides in a typical phagolysosome that fuses with vesicular traffic from endocytic pathways whereas the *C. trachomatis* inclusion appears to be disconnected from the endocytic and lysosomal pathways. We have made two significant advances in our understanding of the chlamydial inclusion. A novel experimental approach has identified secreted chlamydial polypeptides that modify the inclusion membrane. The inclusion membrane appears to be host derived as demonstrated by direct trafficking of a sphingolipid probe through the Golgi apparatus to the cell wall of the chlamydiae themselves. Sphingomyelin, endogenously synthesized from C₆-NBD-ceramide, was specifically transported to the inclusion and incorporated into the cell wall of the intracellular chlamydiae. The chlamydial inclusion thus appears to be integrated into an exocytic pathway which delivers endogenously synthesized sphingolipids from the Golgi apparatus to the chlamydial inclusion. Endocytosed chlamydiae are trafficked to the Golgi region relatively rapidly and begin to acquire sphingolipids from the host within a few hours following infection. The transport of NBD-sphingolipid to the inclusion is reduced by brefeldin A, depletion of ATP pools, or by incubation at 20°C suggesting an active, vesicle-mediated process. Other Golgi-specific markers including various lectins and resident protein markers of the *trans*-Golgi demonstrate the close association of the Golgi apparatus with the chlamydial inclusion although none of these markers appear to be incorporated into the inclusion membrane. Collectively, the data suggest that the chlamydial inclusion occupies a site distal to the Golgi apparatus situated such that it receives vesicular traffic from the Golgi normally bound for the plasma membrane. Although, chlamydial infection disrupts exocytosis of sphingolipid, glycosylation and exocytosis of secreted proteins does not appear to be significantly affected. The chlamydial inclusion thus appears to represent an aberrant exocytic vesicle derived from

the *trans*-Golgi; a probable means of modifying this vesicle is the insertion of chlamydia-specified proteins. Convelescent sera were used to identify antigens present in chlamydia-infected tissue culture cells, but absent from the infectious elementary body (EB). Adsorption of convalescent sera with EBs produced a reagent with dominant reactivity toward the inclusion membrane, with no apparent reactivity toward EBs or RBs. An expression library of *C. psittaci* (strain GPIC) DNA was therefore screened with this convelescent sera to identify a recombinant protein not recognized by antisera against formalin-fixed elementary bodies (EB). The first of what appear to be multiple parasite-specified polypeptides in the inclusion membrane has been cloned, sequenced, and expressed. This sequence was amplified and fused to the *malE* gene of *E. coli*. Antisera against the resulting fusion protein were used to demonstrate that p39 was localized to the inclusion membrane of infected HeLa cells. Taken together, the results imply that the chlamydial inclusion represents a Golgi-derived vesicle modified by the insertion of chlamydia-specified proteins.

Molecular Pathogenesis Section: The Molecular Pathogenesis Section is under the direction of Dr. Witold Cieplak, Jr. The Section is involved in the study of the structure function relationships and physiology of bacterial NAD-dependent, ADP-ribosylating toxins, particularly *Escherichia coli* heat-labile enterotoxin (LT) and cholera toxin (CT). The recent publication of the X-ray crystal structure of LT, a close relative of CT, has facilitated the investigation of the potential involvement of various residues in a predicted NAD-binding cleft. The refined crystal structure has shown that arginine 7, a residue section members previously showed to be involved in enzymatic activity, lies within the predicted NAD⁺-binding site. More recently, section members have altered various residues by site-directed mutagenesis, including histidine 44, serine 61, and glutamic acids 110 and 112 using multiple isoonic or isosteric substitutions. These residues are predicted to lie within or be vicinal to the substructure or cleft presumed to be the NAD⁺-binding site. While virtually all substitutions at these residues are capable of dramatically reducing enzymatic activity, retention of gross conformation, as judged by sensitivity to limited trypsinolysis, is dependent upon the type of substitution introduced. Detailed kinetic analyses have revealed a specific catalytic role for glutamic acid 112, perhaps involving the formation of an oxocarbonium-like intermediate. Similar analyses point to a structural role for serine 61 in maintaining the geometry of the active site by hydrogen bonding. Mutations that have been judged to result in reductions of enzymatic activity but no detectable alterations in overall conformations are being introduced into the gene for the holotoxin to assess their effects toxin assembly, cytotoxic/enzymatic activity, and ability to potentiate mucosal immune responses to both themselves and heterologous antigens. Members of the Section have begun analyses of potential residues involved in the NAD-binding site using photolabeling techniques. These studies have been facilitated by the development of an FPLC purification scheme that results in preparations of recombinant A subunits and analogs of greater than 95% purity. These findings will have important implications in establishing the precise nature of the interactions between the toxins, ARF, and the target G proteins. Crystallization studies are also in progress using the purified active subunit that has been chemically modified to render it

homogeneous and "lock" it in the enzymatically active conformation. The crystal structure of such a molecule will provide a considerably more accurate picture of the active site of the A subunit of LT. Studies of the intracellular trafficking of LT and CT that lead to cell toxicity also continue to be pursued. These studies have shown that LT gains entry into eukaryotic cells via a vesicular transport mechanism that, although, brefeldin A sensitive, does not depend upon an intact main Golgi stacks. The relevant intoxication pathway is also independent of endosomal acidification and microtubule function. Indeed, agents which disrupt these functions markedly potentiate toxic activity in certain cell lines. These finding have led to a model of toxin trafficking that involves movement from an early or intermediate endosomal compartment to the *trans*-Golgi region where either cytosolic translocation or ADP-ribosylation of heterotrimeric G proteins occurs, thus avoiding normal degradative pathways.

ADMINISTRATIVE REPORT

The LICP is divided into four sections: (i) the Host-Parasite Interactions Section (ii) the Immunology of Intracellular Parasites Section, (iii) the Molecular Pathogenesis Section, and (iv) the Mycobacterium Section. The Host-Parasite Section is headed by Dr. Ted Hackstadt, the Immunology Section by Dr. Harlan Caldwell, the Molecular Pathogenesis Section by Dr. Witold Cieplak and the Mycobacterium Section by Dr. Clifton E. Barry, III. Scientists working in the Host-Parasite Section include: Dr. Paul Policastro (Senior Staff Fellow), Dr. Richard Garzon (IRTA), Dr. Robert Heinzen (IRTA), Dr. Daniel D. Rockey (IRTA), Dr. Marci Scidmore (IRTA). Scientists working in the Immunology Section include: Dr. Hua Su (Staff Scientist), Dr. Richard Morrison (Microbiologist), Dr. Todd Cotter (IRTA), and Dr. Scott Manning (Staff Fellow). Drs Morrison, Cotter, and Manning have accepted faculty positions at the University of Alabama, Wisconsin, and Montana, respectively, this past FY. Dr. Linda Perry, a cellular immunologist and recent DVM graduate has been recruited to the Immunology Section and will join the laboratory in November of 1995. Members of the Mycobacterial Section include: Dr. Katie George (IRTA), Dr. Khisimu Mdluli, (Visiting Fellow), and Dr. Ying Yuan (Visiting Associate). Members of the Molecular Pathogenesis Unit include: Dr. Christopher Grant (Visiting Associate).

Summer students working in LICP include: Brian Chesebro (Summer IRTA), Eric Johansen (Summer IRTA), Sarah Young (Summer IRTA), and Jami Reeves (Student IRTA). The following people were invited, by LICP staff, to present seminars: Dr. Joseph Igietseme, University of Arkansas for Medical Sciences, Little Rock, Arkansas, Dr. Robert C. Brunham, University of Manitoba, Department of Medical Microbiology, Winnipeg, Manitoba, Canada, Dr. Tom Jerrells, Louisiana State University, Dr. Camella Bailey, University of North Carolina, Dr. Hiroshi Nikaido, University of California, Berkeley, Dr. Daniel A. Portnoy, University of Pennsylvania, Philadelphia, PA., Dr. Raphael Valdivia, Stanford University, CA., Dr. Francis Nano, University of Victoria, British Columbia, Canada.

HONORS AND AWARDS

NIH Honor Awards: None

Journal Editorial Boards:

H. Caldwell - Infection and Immunity

W. Cieplak - Infection and Immunity

Ad Hoc Reviewers:

H. Caldwell - Cell
Journal of Bacteriology
Journal of Infectious Diseases
Journal of Immunology
Proceedings of the National Academy of Sciences
Microbiol Pathogenesis

W. Cieplak - Infection and Immunity
Journal of Immunology

T. Hackstadt - Proceeding of the National Academy of Sciences, USA
Journal of Bacteriology
Molecular Microbiology
Journal of Infectious Diseases
Journal of Medical Microbiology

Professional Posts:

H. Caldwell - Faculty affiliate, Division of Biological Sciences (Microbiology),
University of Montana, Missoula, Montana.

Invited Lectures and Participation in Meetings and Symposia:

C. Barry - Molecular Mechanisms in Tuberculosis, Keystone Meeting, Tamarron,
CO.
Multi-Drug-Resistant Tuberculosis Meeting, Vail, CO.
PathoGenesis Corporation, Seattle, WA.
Thirtieth Joint U.S.-Japan Conferences, Ft. Collins, CO.

H. Caldwell-	NIAID, Vaccine Advisory Board, Bethesda, Maryland United Biomedical, New York University of Goteborg, Goteborg, Sweden ISSTDR, New Orleans, Louisiana
T. Cotter -	American Society for Microbiology, Washington, D.C.
T. Hackstadt -	Seminar, Chapel Hill, University of North Carolina Northwest Branch of ASM/Western Branch of the Canadian Society of Microbiologists, University of Montana, Missoula, Montana ISSTDR, New Orleans, Louisiana
R. Heinzen -	American Society for Microbiology, Washington, D.C.
K. Mdluli	American Society for Microbiology, Washington, D.C.
P. Policastro-	American Society for Microbiology, Washington, D. C.
D. Rockey	American Society for Microbiology, Washington, D.C. Northwest Branch of ASM/Western Branch of the Canadian Society of Microbiologists, Missoula, Montana
H. Su	ISSTDR, New Orleans, Louisiana

Other Activities:

H. Caldwell -	NIAID Study Section, Bacteriology and Mycology, Ad hoc member National Advisory Allergy and Infectious Diseases Council NIAID Promotion and Tenure Committee
T. Hackstadt -	Medical Research Council of Canada, Ad hoc reviewer National Science Foundation, Ad hoc reviewer NIAID, RML, Biosafety Committee, Chair and Institutional Biosafety Officer NIAID, RML, Safety Committee NIAID, RML, Library Committee

C. Barry NIAID, RML, Biosafety Committee

D. Rockey NIAID, RML, ACUC Committee

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00216-15 LICP
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Chlamydial Vaccine Development		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	H.D. Caldwell	Chief LICP, NIAID
Others:	H. Su	Staff Scientist LICP, NIAID
	W. Cieplak	Sr. Staff Fellow LICP, NIAID
	J. Simmons	Bio. Lab Tech. LICP, NIAID
COOPERATING UNITS (If any) Dr. Andrew Murdin, Connaught Laboratories, Willowdale, Ontario, Canada		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Immunology of Intracellular Parasites		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 0.6	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided...)		
<p> <u>Chlamydial trachomatis</u> is a leading cause of sexually transmitted diseases worldwide for which there is no effective vaccine. The objective of this project is the design of recombinant vaccines for the prevention of infections caused by <u>Chlamydia trachomatis</u>. Chlamydial infections are restricted to the oculogenital mucosae and are caused by multiple chlamydial serovars. Local antibody (slgA) is thought to play an important role in protection against chlamydial colonization and infection of mucosal epithelial cells. The goal of this work is to generate a subunit or recombinant chlamydial vaccine capable of evoking broadly cross-protective anti-chlamydial neutralizing IgA antibodies at the oculogenital mucosae. The chlamydial major outer membrane protein (MOMP) is the principle neutralizing antigen on the chlamydial surface. Several approaches are being used to target rMOMP or protective MOMP epitopes to evoke slgA anti-chlamydial neutralizing antibodies. These include incorporation of the epitopes as gene fusions with the B subunit of <u>E. coli</u> enterotoxin (LT), the construction of recombinant polioviruses expressing MOMP epitopes as gene fusions with the poliovirus major capsid protein VP1, and encapsulation of MOMP into microspheres. Our findings show that each of these approaches is capable of eliciting high titered serum anti-chlamydial neutralizing antibodies (IgG) following parenteral immunization however none of these systems has been effective in evoking local slgA responses. Future studies will focus on the generation of recombinant LT-MOMP immunogens designed to both optimize the mucosal adjuvanticity of LT and the mucosal immune response against the MOMP. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER ZO1 AI 00519-08- LICP
NOTICE OF INTRAMURAL RESEARCH PROJECT		
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunopathogenesis of Chlamydial Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and Institute affiliation)		
PI:	R.P. Morrison	Microbiologist LICP, NIAID
Others:	K. Lyng	Chemist LICP, NIAID
COOPERATING UNITS (If any) Univ. Toronto, Ontario, Canada (R. Innam); Univ. Wisconsin Med. Sch., Madison, WI (G.Byrne, W.Beatty); New York Hosp.-Cornell Med. Ctr., New York, NY (S. Witkin); University of Montana, Division of Biological Science, Missoula, Montana (Y. Ying). Indiana Univ. School of Medicine, Indianapolis, Indiana (R.Jones, J.Arno).		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Immunology of Intracellular Parasites		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)		
PROJECT TERMINATED		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00552-07 LICP
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunopathobiology of bacterial toxins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	W. Cieplak, Jr.	Unit Head LICP, NIAID
Others:	C.R. Grant	Visiting Associate LICP, NIAID
	R. Messer	Microbiologist LICP, NIAID
	D.J. Mead	Microbiologist LICP, NIAID
COOPERATING UNITS (if any) Boyd E. Haley (University of Kentucky); Rockford K. Draper (University of Texas at Dallas); D. Scott Manning (University of Montana); John Nedrud (Case Western Reserve University); Wim Hol (University of Washington); James Cowell (Praxis Biologics); Harlan Caldwell (LICP, NIAID).		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Molecular Pathogenesis Unit		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	0.9	1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided...)		
<p>Analyses of mutant analogs of <u>Escherichia coli</u> heat-labile enterotoxin (LT), a close relative of cholera toxin (CT), have continued. Several residues have been identified by site-directed mutagenic analyses as playing an essential catalytic role in the ADP-ribosyltransferase activity of these toxins. Substitutions at these positions appear to allow retention of native conformation. These mutations, in conjunction with other previously described substitutions may be a suitable target for the generation of, multiply substituted mutant proteins that are devoid of toxic/enzymatic activity and that retain native conformation. The wild-type LT and mutant analogs are also being used as delivery systems for heterologous antigens and peptides, particularly those from <u>Chlamydia trachomatis</u>, to exploit the effectiveness of LT in potentiating mucosal immune responses. We have also continued to investigate the mechanism(s) by which these toxins are internalized and gain access to intracellular G protein substrates. Studies using various metabolic inhibitors indicate some interesting differences in response to LT and CT among various target cell lines or types. The data also indicate that microtubule-mediated processes are not involved in toxin trafficking and indeed enhance their cytotoxic/cytotoxic effects under some circumstances. Other studies indicate that occupation of a non-acidified endocytic compartment is involved in the relevant intoxication pathway. The data collectively are consistent with an internalization pathway that involves the trans-Golgi region or another late endosomal compartment that bypasses lysosomal or other acidified endocytic vacuoles.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00553-06 LICP
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology and Immunology of Pathogenic <u>Campylobacter</u> spp.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	W. Cieplak, Jr.	Unit Head LICP, NIAID
Others:	M.E. Konkel D.J. Mead	IRTA LICP, NIAID Microbiologist LICP, NIAID
COOPERATING UNITS (If any) University of Arizona (Lynn Joens).		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Molecular Pathogenesis Unit		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: center; padding: 10px;"> PROJECT TERMINATED </div> <p>With the departure of the post-doctoral fellow who was responsible for studies in the area of <u>Campylobacter</u> pathogenetic mechanisms, this project was terminated in the Fall/Winter of 1994 so that we could focus our efforts on the studies of bacterial ADP-ribosylating toxins. Prior to that time we identified and characterized an intron-like element in the 23S ribosomal subunit genes of <u>C. jejuni</u>. Additional studies have characterized in vitro phenotypic passage variants of <u>C. jejuni</u>. In addition, two other outer membrane protein genes of <u>C. jejuni</u> were cloned, sequenced, and expressed in <u>E. coli</u>. One protein represents a homolog/analog of peptidoglycan-associated lipoproteins found in several other medically important bacteria (e.g. <u>Haemophilus influenza</u>) and may represent an important immunogenic determinant. The other protein is a major outer membrane protein that is homologous to porins of other Gram negative bacteria. This work has been completed and is in the process of preparation for publication.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00567-05 LICP
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular Biology of Host-Parasite Interactions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T. Hackstadt	Expert LICP, NIAID
Others:	R. Garzon	IRTA LICP, NIAID
	R.A. Heinzen	IRTA LICP, NIAID
	J.D. Sager	Bio. Lab Tech. LICP, NIAID
	D.D. Rockey	IRTA LICP, NIAID
	M.A. Scidmore	IRTA LICP, NIAID
	R.E. Mann	Bio. Lab Tech. LICP, NIAID
	P.F. Policastro	Sr. Staff Fellow LICP, NIAID
COOPERATING UNITS (If any) None		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Immunology of Intracellular Parasites Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.75	5.75	2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The chlamydial inclusion remains largely uncharacterized as to its cellular origins. Neither fluid phase markers nor markers for various stages of the endocytic/lysosomal pathway are associated with the chlamydial inclusion. The mature inclusion therefore appears disconnected from endocytic pathways. However, fluorescent sphingomyelin endogenously synthesized from a Golgi-specific lipid, C6-NBD-ceramide, is translocated from the Golgi apparatus to <u>Chlamydia trachomatis</u> inclusions in a time, temperature, and energy dependent manner where it is rapidly incorporated into the cell walls of the intravacuolar chlamydiae. Approximately one-half of the newly synthesized sphingomyelin in <u>C. trachomatis</u> infected cells is trafficked unidirectionally to the inclusion. The transport of sphingomyelin to the inclusion is direct and specific to the chlamydial inclusion. NBD-sphingomyelin, incorporated into the plasma membrane, is not delivered to the chlamydial inclusion. Other Golgi-specific markers including various lectins and resident protein markers of the <u>trans</u>-Golgi demonstrate the close association of the Golgi apparatus with the chlamydial inclusion although none of these markers appear to be incorporated into the inclusion membrane. A novel experimental approach has identified secreted chlamydial proteins that are inserted into the inclusion membrane. The first of what appears to be multiple chlamydia-specified proteins in the inclusion membrane has been cloned, sequenced, and expressed. Based upon these observations, we suggest that the chlamydial inclusion represents an aberrant vesicle derived from the <u>trans</u>-Golgi network, modified by the insertion of chlamydial proteins, and that inclusion membrane growth may be supported by the interruption of a cellular exocytic pathway. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER ZO1 AI 00631-04 LICP	
PERIOD COVERED October 1, 1994 to September 30, 1995			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Biology of Intracellular Parasites			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	T. Hackstadt	Expert	LICP, NIAID
Others:	P.F. Policastro	Senior Staff Fellow	LICP, NIAID
	D.D. Rockey	IRTA	LICP, NIAID
	R.A. Heinzen	IRTA	LICP, NIAID
	M. Scidmore	IRTA	LICP, NIAID
	R.E. Mann	Bio Lab Tech.	LICP, NIAID
COOPERATING UNITS (if any) None			
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999			
SECTION Host-Parasite Interactions Section			
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892			
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)			
PROJECT TERMINATED			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00672-03 LICP
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mucosal Immunity to Chlamydial Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	H.D. Caldwell	Chief LICP, NIAID
Others:	H. Su	Staff Scientist LICP, NIAID
	K. Feilzer	Microbiologist LICP, NIAID
	J. Simmons	Bio. Lab Tech. LICP, NIAID
COOPERATING UNITS (If any)		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Immunology of Intracellular Parasites		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	1.5	1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)		
<p>Genital tract infections caused by <u>Chlamydia trachomatis</u> are a leading cause of sexually transmitted diseases (STDs). Immunoprophylaxis is one way to control chlamydial caused STDs. Host immunity to chlamydial infections of the genital tract mucosae are poorly understood. This lack of understanding has hampered progress towards the development of an efficacious chlamydial vaccine. The goal of this project is to use a murine model of chlamydial genital tract infection to define those immune mechanisms that are important in acquired immunity to infection and to identify those chlamydial antigens which elicit protective immune responses. By using genetically defined knock out mice deficient in specific immune functions and adoptive immunization experiments we have shown that CD4+ T cells play a significant role in the acquired immune response to chlamydial infection. CD4+ T cells function in providing cognate help for antibody production (Th2) and in cell mediated immunity (Th1). Our data show that chlamydial specific serum or secretory antibody plays only a minor role in acquired immunity to chlamydial infection negating a critical role for chlamydial specific CD4+ Th2 cells in protective immunity. Conversely, Th1 cells are critical for the resolution of chlamydial infection and resistance to re-infection. The mechanism by which CD4+ Th1 cells function and those chlamydial antigens recognized by protective CD4+ Th1 cells are under investigation. Definition of the mechanism(s) by which CD4+ T cell subsets function in protective immunity and characterization of those chlamydial antigens recognized by such subsets will provide useful information for the design of subunit or recombinant vaccines for the prevention chlamydial infection of the genital tract mucosa.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00693-03 LICP
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenic Mechanisms of <i>Mycobacterium tuberculosis</i>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	C.E. Barry	Unit Head LICP, NIAID
Others:	Y. Yuan S. Stewart	Visiting Associate Microbiologist LICP, NIAID LICP, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840-2999		
SECTION Mycobacterial Unit		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided...)		
<p>The overall objective of this work is the development of novel chemotherapeutics and chemotherapeutic targets for the treatment of diseases of Mycobacterial origin including tuberculosis, leprosy, and <i>M. avium-intracellulare</i> (MAC) infections of AIDS patients. Pathogenic strains of mycobacteria uniformly modify their major mycolic acids by cyclopropanation while non-pathogenic strains do not. We have used this distinction to clone two genes from a pathogenic species (<i>M. tuberculosis</i> (MTB)) into a non-pathogenic species (<i>M. smegmatis</i>(MSMEG)) which cyclopropanate mycolic acids. <u>cma1</u> has been shown to function to cyclopropanate the distal cis double bond in the longer meromycolate branch, while <u>cma2</u> cyclopropanates the proximal double bond. By coexpressing both genes, we have been able to entirely recreate the normal MTB major alpha mycolic acid in MSMEG as well as create two novel mycolates which are hybrids of the MTB and MSMEG structural types by individual expression. Aside from three mycobacterial genes (including a homolog of <u>cma2</u> we have identified from <i>M. leprae</i>) these enzymes are homologous to the only known enzyme with a related function, the <i>E. coli</i> cyclopropane fatty acid synthase. Using these constructs we have shown that cyclopropanation of cell-wall lipids protects MSMEG from oxidative killing, suggesting a potential role in pathogenesis. In addition we have shown by physical measurements that cyclopropanation changes the fluidity and structure of the mycobacterial cell wall and are using the recombinant organisms to allow a definition of the cell wall structure. Surrounding DNA sequences encodes proteins which are clearly involved in mycolic acid biosynthesis and are revealing new clues about the biosynthesis, regulation, and functions of mycolic acids. Since isoniazid, which directly affects mycolic acid biosynthesis, is an effective chemotherapeutic, each of these genes independently represents a potential drug target. We are actively pursuing the development of cell-free activity assays and the syntheses of compounds designed to inhibit these steps as potential chemotherapeutics for the treatment of diseases of mycobacterial etiology.</p>		

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1995 Annual Report
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1994, to September 30, 1995

RESEARCH HIGHLIGHTS

The Laboratory of Microbial Structure and Function (LMSF) studies two microbes that are commonly sexually-transmitted (*Neisseria gonorrhoeae*, gonorrhea; Human Immunodeficiency Virus, AIDS) and several others that employ insect vectors for transmission (tick borne include *Borrelia burgdorferi*, Lyme disease and *Borrelia hermsii*, relapsing fever; flea borne are *Yersinia pestis*, plague). Although this collection of microorganisms displays a diversity of life-styles when inhabiting human hosts, they all show extensive variation in surface-exposed components. Surface variations clearly allow some (e.g. HIV, *B. hermsii*) to evade and survive host immune responses. The pathogenetic relevance of surface variation is not entirely clarified for *B. burgdorferi*, but it clearly relates to this bacterium's survival in both ticks and mammals. Accumulation of hemin on its surface, assures *Y. pestis* of retention in a flea's gut by exploiting processes designed for "tanning" and hardening chitin that lines the flea proventriculus. *N. gonorrhoeae* displays surface variations of extraordinary diversity and high frequencies *in vitro* and *in vivo*; but absence of an animal model limits understanding of the pathobiologic impact; but the genetic events that give rise to variations in surface components of *N. gonorrhoeae* are amenable to dissection and provide sign posts for understanding analogous results in other microbes.

A major thrust of studies in LMSF on surface variability is the aim to probe microbe-host relationships that relate to infection pathogenesis. The diversity of genetic mechanisms that these microorganisms use to vary their surface components and the range of biological consequences that accompany their such variations provide both an opportunity and the environment for extensive cross-talk and collaboration among LMSF staff members.

Joe Hinnebusch and Tom Schwan, in their studies on flea-*Yersinia pestis* interactions, have been examining the pathobiological effects of the *hms* locus of *Y. pestis* which encodes for hemin accumulation in the outer membrane of this bacterium. Organisms that are *hms*⁺, when introduced into the flea with a blood meal from an infected animal, multiply and form a "clot" in the flea proventriculus which is analogous to the esophagus of higher animals. This occludes the proventriculus, prevents subsequent blood meals from entering the flea's midgut, causes "regurgitation" of viable *Y. pestis* into the animal on which a flea is feeding, and results in highly efficient transmission of *Y. pestis* to a new animal host. *Y. pestis* that are *hms*⁻ do not occlude the flea proventriculus and are transmitted inefficiently from flea to animal. Very recent morphologic data suggest that hemin in/on the *hms*⁺ *Y. pestis* outer membrane is cross-linked and "tanned" by highly reactive compounds that function to harden and melanize the chitin in the flea proventriculus. Accordingly, *hms*⁺ *Y. pestis* comes to form and occupy a horny clot in the flea's digestive system by dint of a system that functions in flea development and physiology.

Schwan and Hinnebusch also demonstrated that different serotypes of *Borrelia hermsii* are transmitted by vector ticks to mice at differing efficiencies. This suggests that the changes

in the variable surface protein (Vsp) of *B. hermsii* may have roles in addition to evading the serotype-specific antibody response of an infected mammal host.

Tom Schwan and Patti Rosa refined and extended their observations on differential expression of Outer Surface Proteins (Osp's) by *B. burgdorferi* in tick versus mammals. Production of OspC by *B. burgdorferi* is switched on in the tick midgut that becomes engorged with blood; prior to the blood meal, the spirochetes express OspA but not OspC. *In vitro*, OspC production is switched-on when the spirochetes in culture are shifted from 24°C to 37°C. Expression of OspC may relate both to the migration of *B. burgdorferi* from tick midgut to salivary gland and to establishment of infection in mammals which develop early antibodies to OspC but not OspA.

Patti Rosa and Brian Stevenson have found differential expression of several genes in *B. burgdorferi* in addition to OspC. They have focused mainly on genes and gene products involved in guanosine synthesis/salvage and on outer surface proteins. Preliminary results indicate that five gene products besides OspC exhibit temperature-related differential expression in *B. burgdorferi*. Patti Rosa has also identified an operon in *B. burgdorferi* that is probably involved (by sequence comparison) with homologues of the transmembrane and ATPase components of an oligopeptide permease system (OppBCDF). Rosa is constructing defective versions of these genes and, with an electroporation-transformation system developed recently by Scott Samuels (RMMB), she will introduce them into *B. burgdorferi* to further inquire as to their functions in spirochetes.

Earlier studies by John Swanson established that expression of Opa proteins leads to *N. gonorrhoeae* acquiring a positive surface charge and avidly accreting polyanions such as sulfated polysaccharides and oligo/polynucleotides. Bob Belland and John Swanson found that nonpilated *N. gonorrhoeae* expressing a particular Opa protein in their outer membrane adhere to eukaryotic epithelial cells via a heparan sulfate-containing glycoprotein. Independent corroboration came from the work of Jos van Putten who recently joined LMSF and identified a eucaryotic cell syndecan that functions as receptor for both Opa+ *N. gonorrhoeae* and Opc+ *N. meningitidis*. Swanson finds that large sulfated polyanions have multiple effects on Opa+ *N. gonorrhoeae*, including: resistance to normal human sera, completion of division septa, formation and dissolution of zones of adhesion, extensive outer membrane blebbing. Polycations (e.g. protamine) reverse these polyanion-induced affects. These findings have likely relevance to *N. gonorrhoeae* residing in mucosal sites bathed by glycosaminoglycans and, periodically, by fluids rich in polycationic compounds.

In continuing attempts to understand nonhomologous recombination events that control on/off switching of several *N. gonorrhoeae* genes, Bob Belland has examined switching rates of recombinant *opa* gene in a wide variety of *E. coli* strains with defined genetic defects in DNA replication, repair, and supercoiling. In general, these defects led to increase in switch frequencies and their analogues have been identified in *N. gonorrhoeae*. The methylation portion of a restriction/modification (*ngoX*) system discovered by Belland was found to also switch on/off by nonhomologous recombination. Stuart Hill has identified and purified two small DNA binding proteins (IHF and HU) in *N. gonorrhoeae* and is identifying their target sites. Hill also studies DNA-transformation of *N. gonorrhoeae* and has demonstrated that Opa+ and Opa- organisms display differing propensities to be transformed; he has also found unexpected lethal affects of transformation on *N. gonorrhoeae*.

ADMINISTRATIVE REPORT

Personnel changes in LMSF during FY'95 include the departure of IRTA John Klena to University of Canterbury, Christchurch, New Zealand; Pre-IRTA Cheryl Dooley to University of Iowa to complete her Ph.D.; Visiting Associate Vladimir Tolstikov to Lajos Kossuth University, Debrecen, Hungary; and Medical Officer Seth Pincus and Visiting Fellow Hua Fang to Montana State University, Bozeman. Recruitment includes the transfer of Visiting Associate Christopher Grant from LICP and the arrival of Josephus P. M. van Putten (Max-Planck-Institute für Biologie, Tübingen, Germany) as a Visiting Scientist, and Martine Bos (University of Leiden, The Netherlands) as a Visiting Fellow. Summer IRTA students were Mark Fisher (Idaho State University, Pocatello), Lynn Race (Montana State University, Bozeman), Angela Rogers (Manchester College, North Manchester, IN), and Tara Wehrly (Washington University, St. Louis, MO). Seminars were given by a number of outside guests: Jacques Mahillon (Unite de Genetique-UCL, Louvain-La-Neuve, Belgium), Uli Munderloh (University of Minnesota, St Paul), Michael Chaussee (University of Oklahoma Health Sciences Center, Oklahoma City), Eric Milner (Virginia Mason Research Center, Seattle, WA), Edward Morgan (Scripps Research Institute, La Jolla, CA), Stephen Thomas (University of Victoria, Victoria, BC), Michael Hubank (Yale University, New Haven, CT), Bill Barnett (Utah State University, Logan), and Stephen Barthold (Yale Medical School, New Haven, CT).

HONORS AND AWARDS

Journal Editorial Boards:

- S. Pincus - Medical advisory board for Arthritis Today
T. Schwan - Journal of Clinical Microbiology

Manuscripts were reviewed by LMSF staff for the following journals: American Journal of Medicine, American Journal of Tropical Medicine and Hygiene, Applied and Environmental Microbiology, Canadian Journal of Microbiology, Cell, Clinical Infectious Diseases, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of Experimental Medicine, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Medical Entomology, Journal of Molecular Biology, Journal of Rheumatology, Journal of Spirochetal and Tick-Borne Diseases, Journal of Wildlife Diseases, Microbial Pathogenesis, Microbiology, Molecular Microbiology, New England Journal of Medicine, Plasmid, Proceedings of the National Academy of Sciences USA, Science, and Trends in Microbiology.

Professional Posts:

- S. Pincus - Adjunct Associate Professor of Internal Medicine, University of Utah,
Salt Lake City, UT

Invited Lectures and Participation in Meetings and Symposia:

- H. Fang - Cold Spring Harbor Retroviruses Meeting, New York
- S. Pincus - Meeting on the Development of AIDS Vaccines, Bethesda, MD
Strategies for Managing STDs in Family Planning Settings Symposium,
Baltimore, MD
Fourth International Symposium on Immunotoxins, Myrtle Beach, SC
- P. Rosa - AAAS Annual Meeting, Atlanta, GA
Tulane University Medical Center, New Orleans, LA
Workshop on Population Biology, Evolution and Control of Infectious
Disease, CDC, Atlanta, GA
- T. Schwan - Cold Spring Harbor Banbury Center, New York, Molecular Biology of Lyme
Disease Spirochetes
Northwestern Branch, American Society for Microbiology, University of
Montana, Missoula, MT
- B. Stevenson - Cold Spring Harbor Banbury Center, New York, Molecular Biology of Lyme
Disease Spirochetes
- J. Swanson - University of Texas Southwest Medical School, Dallas, TX
University of Texas, San Antonio, TX
- J. van Putten - Convener of Gordon Conference on Molecular Mechanism of Microbial
Adhesion, Newport, RI

Other Activities:

- R. Belland - NIAID, RML, Institutional Biosafety Committee
- S. Hill - NIAID, RML, Library Committee
- J. Hinnebusch - Biology of Disease Vectors Summer Course, Colorado State University,
Fort Collins, CO
- M. Niebylski - Biology of Disease Vectors Summer Course, Colorado State University,
Fort Collins, CO
- S. Pincus - State of Montana Advisory Committee for Institutional Development Award
HIV Peer Review Panel for the American Institute of Biological Sciences,
Washington, DC
NIAID, RML, Institutional Biosafety Committee
- P. Rosa - *Ad Hoc* Review Committee, Bacteriology and Mycology-1 Study Section,
NIAID
Program Committee, 1996 International Meeting on Lyme Borrelia
NIAID, RML, Animal Care and Use Committee
- T. Schwan - Council Member, American Committee of Medical Entomology (1991-1995)
President, International Northwestern Conference on Diseases of Nature
Communicable to Man
NIAID, RML, Safety Committee
NIAID, RML, Library Committee
- J. Swanson - Review Panel for the Postdoctoral Research Fellowships for Physicians
program, Howard Hughes Medical Institute, Chevy Chase, MD
Reviewed Merit Review Application, Department of Veterans Affairs,
Livermore, CA
- K. Tilly - *Ad hoc* reviewer for International Science Foundation grant
NIAID, RML, EEO Committee

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00193-16 LMSF

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: J. Swanson Chief LMSF, NIAID

Others: J. Klena IRTA Fellow LMSF, NIAID
 J. M. Wilson Biologist LMSF, NIAID

COOPERATING UNITS (if any)

E. C. Gotschlich (Rockefeller University), F. S. Hayes (RMLMB), and D. Dorward (RMLMB)

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gonococci that express Opa proteins generally acquire positive charge which derives from these outer membrane proteins presenting clusters of excess basic amino acids (lysine, arginine, histidine) on their surfaces. Production of Opa proteins by gonococci, as is typical in gonorrheal urethritis of males, endows the bacteria with abilities to adhere to and be ingested by epithelial cells whose heparan sulfate-bearing glycoproteins have been shown to act as "receptors" for Opa+ gonococci. Polyanionic polymers such as heparin, dextran sulfate, and DNA accrete on surfaces of Opa+ gonococci with several consequences depending on their size and charge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00480-10 LMSF

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogen-Arthropod Interactions of Vector-borne Diseases Affecting Public Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	T. G. Schwan	Microbiologist	LMSF, NIAID
Others:	J. Hinnebusch	IRTA Fellow	LMSF, NIAID
	M. Niebylski	IRTA Fellow	LMSF, NIAID
	R. H. Karstens	Bio Lab Tech (Micro)	LMSF, NIAID

COOPERATING UNITS (If any)

Ken Gage (CDC); Alan Barbour (UTHSC); Blanca Restrepo (UTHSC); Donald Anderson (Sacred Heart Hospital); Robert Perry (U Kentucky)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objectives of this project are to use molecular and classical approaches to investigate pathogen-arthropod interactions of vector-borne agents causing diseases of human importance in the United States. Most of our effort has concentrated on *Yersinia pestis*, the causative agent of bubonic plague, *Borrelia hermsii*, an agent of tick-borne relapsing fever, and spotted fever group rickettsiae.

We examined the role of the *hmsHFR* locus of *Y. pestis* in infection and subsequent blockage of fleas. The *hmsHFR* locus is essential for hemin storage (*Hmsa*+) in the outer membrane and formation of pigmented colonies on hemin and Congo red agars at 26-30°C. Oriental rat fleas (*Xenopsylla cheopis*) were fed blood containing 5x10⁸ bacteria per ml of one of 6 strains of *Y. pestis*, using an artificial feeding device. Fleas fed a blood meal infected with *Hms*+ strains exhibited high mortality (41-63% after 4 weeks) and blockage of the midgut (37-42%). Fleas fed uninfected blood, or blood infected with either of 2 *Hms*-mutants showed normal mortality (5-13%) and no blockage. High mortality and blockage were restored when an *Hms*- strain was transformed with a plasmid containing the intact *hms* locus. These data suggest that hemin storage in the outer membrane is essential for *Y. pestis* to block the midgut of the rat flea, which is a critical event leading to efficient transmission of plague.

Tick-borne relapsing fever of humans in the western United States is presently known to be caused by two or three closely related species of *Borrelia* spirochetes transmitted by Argasid ticks in the genus *Ornithodoros*. How these spirochetes vary antigenically in their tick vector, and how different serotypes of the spirochete affects tick infection and transmission, are unknown. During the last year we infected two cohorts of nymphal *Ornithodoros hermsi* ticks with different serotypes of *B. hermsii*. The first group of ticks was infected with serotype 7, and the second group of ticks was infected with serotype 8. The ticks were held at 27°C until they had molted to the third nymphal stage and were again ready to feed. Single ticks from each group were allowed to feed on individual mice and then the peripheral blood of the mice was examined daily for 14 days to determine if ticks had transmitted spirochetes. A total of 95 ticks was fed on individual mice: 16 of 46 ticks infected with serotype 7 transmitted spirochetes (35%) while only 2 of 49 ticks infected with serotype 8 transmitted spirochetes (4%). This is the first demonstration that the frequency of tick transmission of *B. hermsii* is influenced by the serotype infecting the ticks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00492-09 LMSF

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others:	J. Hinnebusch	IRTA Fellow	LMSF, NIAID
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COOPERATING UNITS (if any)

Joe Piesman (CDC); Alan Barbour (UTHSC); Patricia Rosa (LMSF); Mark Klempner (Tufts-NEMC)

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TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lyme disease is the most prevalent arthropod-borne disease of humans in the United States and many other countries throughout Europe and Asia. The objectives of this project are to (1) use recombinant DNA techniques to express specific antigens of *Borrelia burgdorferi* to improve the serodiagnosis of Lyme disease, (2) characterize at the molecular level, isolates of the Lyme disease spirochete from a wide range of biological and geographical sources, and (3) examine adaptive molecular responses produced by *B. burgdorferi* during infection in ticks. During the last year, we directed most of our efforts towards the last objective.

We examined the differential expression of outer surface proteins (Osps) by spirochetes in ticks. Spirochetes in the unfed nymphal ticks' midgut were examined by indirect immunofluorescence (IFA) for the presence of OspA or OspC. Only OspA was detected on spirochetes in these ticks that had not yet fed on blood. We examined antisera from mice infected with spirochetes by tick-bite or by inoculation of midguts from partially engorged, infected ticks. Antisera from all mice had strong antibody responses to OspC but not to OspA, indicating that *B. burgdorferi* produced OspC, but little or no OspA, during early infection in mammals. Spirochetes were grown at 24°C and 37°C and then examined by SDS-PAGE and immunoblot. Spirochetes continued to produce OspC when grown at 37°C but very little at 24°C, demonstrating that the synthesis of OspC was regulated, at least in part, by temperature. Ticks attached and feeding would experience both an increase in temperature and the influx of fresh blood into the midgut where spirochetes reside. Midguts from infected *I. scapularis* nymphs were examined immediately after the ticks had engorged in 3 days on normal mice. In contrast to the spirochetes in unfed ticks, spirochetes in all of the tick smears stained for OspC were positive, demonstrating that during tick feeding spirochetes in the midgut synthesized OspC. However, the incubation of infected, unfed ticks for up to 6 days did not stimulate the spirochetes to produce OspC. This change on the spirochete's surface in the feeding tick, controlled in part by temperature and blood, has relevance to both vaccine development and serodiagnosis of Lyme disease. To our knowledge, this is the first time that a tick-borne bacterial pathogen has been shown to alter its expression of a specific surface component during tick feeding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00516-08 LMSF

PERIOD COVERED

October 1, 1994, to August 31, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoglobulin Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	R. L. Cole	Chemist	LMSF, NIAID
	R. Ireland	Microbiologist	LMSF, NIAID

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5.05

PROFESSIONAL:

3.6

OTHER:

1.45

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this work is to study the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy.

A. Therapeutic systems. 1. Antibodies to surface antigens of group B streptococci (GBS) have been demonstrated to have protective efficacy in a model of neonatal sepsis. We have identified colony opacity variants of GBS, studied their interactions with host defenses, and shown that opacity differences are even more marked in clinical isolates than in the laboratory strains previously analyzed. 2. The efficacy of anti-HIV antibodies coupled to ricin A-chain has been studied *in vitro*. Antibodies directed against different envelope epitopes have been tested. Biological variants of HIV that escape killing with these immunotoxins and CD4-PE40 have been identified. The phenotype of the cells carrying these HIV has been studied, and the molecular mechanisms of immunotoxin escape have been evaluated. Immunotoxins are being tested *in vivo* in well-studied animal systems. 3. Evaluation of the anti-HIV antibody response in subjects exposed to the IIIB/LAV isolate of HIV. Subjects include humans and chimpanzees infected with the virus or immunized with envelope subunits.

This project terminated August 31, 1995.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00549-07 LMSF

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Variation and Adaptation in *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	P. A. Rosa	Senior Staff Fellow	LMSF, NIAID
Others:	K. L. Tilly	Senior Staff Fellow/Bio Lab Tech	LMSF, NIAID
	B. Stevenson	IRTA Fellow	LMSF, NIAID

COOPERATING UNITS (if any)

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 S. Samuels, RMLMB

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3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our broad objective is to understand the means by which *Borrelia burgdorferi* (Bb) establishes an infection:transmission cycle between the tick vector and mammalian reservoir host, both of which are needed to maintain the spirochete in nature.

1. Outer surface protein variation. The outer membrane of Bb contains several abundant proteins (Osp)s that vary in size and expression and are of unknown function. It is likely that the different Osp)s confer distinct properties on the spirochete that are pertinent to the different environments in which it must survive. Collaborative studies with Dr. Schwan have shown that OspC is detectable on spirochetes in the midgut of infected ticks only after a bloodmeal, and that an increase in temperature is in part responsible for increased OspC synthesis. Dr. Stevenson has documented that at least 5 other proteins are differentially synthesized as a consequence of culture temperature. Dr. Stevenson has analyzed the 5' flanking sequence of the *ospC* gene from multiple strains to identify conserved promoter elements. Fisher, Stevenson and Tilly have initiated studies using both genetic and biochemical approaches to identify components that alter *osp* promoter activity. Stevenson, Tilly and Rosa are attempting to transform Bb and inactivate *osp* genes and other genes of interest by integrating selectable markers. Such knockout mutants are a critical component of future studies to determine the roles of various proteins, including Osp)s, in the infectious cycle of Bb.

2. Plasmid structure and replication. Understanding the structure and replication of the unusual linear and circular *Borrelia* genome is of intrinsic interest and practical merit. Dr. Tilly has continued her studies of a Bb HU/IHF homolog. She and Dr. Samuels (RMLMB) have developed a purification scheme and used a gel shift assay to identify protein fractions that bind to a telomeric DNA fragment that contains a putative IHF binding site. Tilly and Rosa have developed a strategy to analyze the site at which telomeric sequences recombine. Rosa and Tilly have extended their analysis of the structure and function of the unique plasmid-encoded Bb IMP dehydrogenase gene homolog (*guaB*) by comparing it to the closely related, but more typical, homolog in *B. hermsii*. Previous studies have identified a gene homolog of a peptide binding protein adjacent to *guaB* on the 26-kB circular plasmid. Dr. Rosa has extended this study by identifying a chromosomal locus homologous to an oligopeptide permease system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00608-05 LMSF

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression and Phase Variation of Gonococcal *opa* Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	J. Swanson	Chief	LMSF, NIAID
	J. Carlson	IRTA Fellow	LMSF, NIAID
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COOPERATING UNITS (if any)

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3.8

PROFESSIONAL:

2.8

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Human infections with *Neisseria gonorrhoeae* are characterized by the variability of the pathogen during the course of disease. We have studied one family of outer-membrane proteins (the Opa or "opacity-related" proteins) which have been shown to be expressed in vivo during natural and human challenge infections. We have focussed on the genetic mechanisms used by the organism to vary expression of this protein family and the biological properties associated with expression.

A common mechanism drives the expression state changes ("phase variation") of the *opa* multigene family which serves as a paradigm for other genes and gene families which utilize similar means of genetic variation. Changes in the number of short DNA repeats within the coding region of the individual genes alter their ability to be fully translated into mature proteins ("translational-frameshifting"). We have identified a number of cellular processes which influence the ability of the bacterium to control the rate of change in these repetitive sequences. Specific genes involved in these processes have been isolated from *N. gonorrhoeae* and their role in phase variation is being studied.

The variably expressed Opa proteins have previously been described as imparting a wide variety of biological phenotypes (generally involving increased adherence to human cells). We have focussed our studies on the role of Opa protein expression in resistance to early (i.e. non-immune) host defence mechanisms. Specifically the influence of Opa protein expression on the ability of bacterial cells to resist killing by human complement (in normal human serum, NHS) and human PMNs (in the presence and absence of NHS).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00694-02

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Analysis of the Gonococcus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	J. M. Wilson	Biologist	LMSF, NIAID

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PROFESSIONAL:

1.5

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Homologous recombination impacts profoundly on the biology of *Neisseria gonorrhoeae* either through the rearrangement of specific gene sequences that encode surface antigens, or, by allowing unfettered exchange of chromosomal DNAs via horizontal transmission *in vivo*. Thus, recombination not only shuffles specific gene sequences, but also provides a means to disseminate the variant genetic information throughout a population. Consequently, large variant populations exist, that make therapeutic intervention difficult and impair the efficacy of many vaccine candidates. Therefore, it is the goal of this work to define more precisely, 1) the molecular mechanisms involved in the rearrangement of specific chromosomal loci (e.g. *pilE* the gene encoding the surface-exposed pilin polypeptide); and 2) examine the genetic limitations that may exist in the exchange of chromosomal DNAs between cells. It is hoped that these investigations may allow for novel therapeutic interventions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00724-01 LMSF

PERIOD COVERED

April 1, 1995, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Cross-Talk Between the Pathogenic *Neisseriae* and Human Mucosal Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: J. P. M. van Putten Visiting Scientist LMSF, NIAID

Others: R. L. Cole Chemist LMSF, NIAID
R. Ireland Microbiologist LMSF, NIAID

COOPERATING UNITS (if any)

Prof. T.F. Meyer, Max-Planck Inst. Biol, Tuebingen, Germany; H. Smith and J. Cole, Univ Birmingham, UK; Prof. M. Frosch, Mediz. Hochschule Hannover, Germany; Dr. P. Buck, Montana Eye Bank, Missoula, MT; Dr. F. de Vries, Univ Amsterdam, Netherlands

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OTHER:

0.55

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies were initiated to unravel the function of surface variation of *Neisseria gonorrhoeae* and *Neisseria meningitidis* in the establishment of neisserial disease. Spontaneous variants and genetically defined recombinant strains differing in the expression of several major variable surface components - opacity outer membrane protein (Opa), Opc (meningococci only), lipopolysaccharide (LPS) and polysialic capsule (meningococci only), showed antigen variation-dependent binding to previously identified heparan sulfate containing mucosal cell-surface receptors. Adherence to these receptors is a first step towards Opa/Opc-dependent uptake of the bacteria by the mucosal cells. Confocal microscopy showed that this 'invasion' into host cells was associated with a reorganization of the host cell actin cytoskeleton network and required phosphorylation of host cell tyrosine residues. Bacterial ligands for these receptors were identified for a number of clinical bacterial isolates. The functionality of the ligands (Opa/Opc), however, largely depended on the environmental conditions and the composition of the surface polysaccharides. Incorporation of sialic acids into the lipopolysaccharide hampered Opa protein function.

LABORATORY OF MOLECULAR MICROBIOLOGY

1995 Annual Report

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LABORATORY OF MOLECULAR MICROBIOLOGY

SUMMARY

When it was originally established in 1981, the staff scientists of the Laboratory of Molecular Microbiology (LMM) investigated the structure, function and regulation of a diverse group of microorganisms including RNA and DNA viruses, aerobic and anaerobic bacteria and mycoplasmas. Currently, the principal focus of LMM scientists is murine and primate retroviruses although small programs involving mycoplasmas and mouse genetics still exist. The principal area of research activity continues to be studies of the human immunodeficiency virus (HIV). Fundamental investigations of viral gene regulation, protein structure and function, and particle assembly are integrated with studies of the determinants of immunologic protection against HIV and viral pathogenesis.

BIOLOGY OF HIV INFECTIONS

Rapid induction of apoptosis by cell-to-cell transmission of HIV. The role of programmed cell death (apoptosis) in HIV-infected cells has been investigated using a synchronous cell-to-cell transmission model. In this system, cocultivation of chronically infected H9 cells (H9IIIB) with uninfected H9 cells results in complete HIV reverse transcription within 4-6 h and new extracellular virion production within 16 h of cocultivation. The onset of the oligonucleosomal DNA degradation pattern characteristic of apoptosis was detected within 12 h of cocultivation. The kinetics of apoptosis induction were more rapid in cell-to-cell HIV transmission than in cell-free HIV infections, where apoptosis was not detected until at least 100 h after infection. Apoptosis required Env-CD4 interactions but not new HIV DNA synthesis, and occurred in single cells and in cell-cell fusions. Syncytia undergoing apoptosis continued to express the anti-bcl-2 antigen, suggesting that bcl-2 did not prevent apoptosis under cocultivation conditions. Apoptosis was also detected in cocultivations of H9IIIB cells with uninfected peripheral blood lymphocytes from HIV seronegative humans, chimpanzees, and rhesus macaques, demonstrating that induction of apoptosis was not restricted to lymphocytic cell lines or human lymphocytes. Apoptosis was also observed in cocultivations of H9IIIB cells with uninfected differentiated monocytes, even though the virus produced by H9IIIB cells does not infect monocytes. This result suggests that an apoptotic signal may be initiated by CD4-Env interactions which do not otherwise result in productive HIV infections. (Maldarelli and Martin)

Analysis of early events of HIV-1 replication preceding integration. Although HIV infection occurs rapidly in activated T-cells and monocyte-derived macrophages, infection of quiescent CD4+ lymphocytes proceeds only through the reverse transcription step; new HIV protein synthesis is not detected and no extracellular virions are produced. Cocultivation of H9IIIB cells with unstimulated peripheral blood lymphocytes resulted in the production of full-length copies of linear DNA, but no new HIV virion production and the rapid induction of apoptosis similar to, and perhaps more rapid than, the rate of apoptosis induced in H9IIIB-H9 cocultivations. This

finding suggested that rapid cell death may in part account for the absence of new HIV virion production in these cultures. To identify reagents useful in dissecting early events in HIV replication, the effect of the drug cyclosporin A (CSA), which blocks cyclophilin incorporation into virions and inhibits spreading HIV infections was investigated. Pretreatment of H9 and H9IIB cells with CSA inhibited HIV reverse transcription 10 to 100 fold. Only minor (less than 2-fold) decreases in the numbers of cell fusions were detected using a novel, FACS-based fusion assay, suggesting CSA blocked HIV replication after cell-to-cell fusion, perhaps at the level of virion uncoating. (Maldarelli, Willey, and Martin)

REGULATION OF HIV GENE ACTIVITY

Binding of transcription factors downstream of the HIV-1 promoter. A previous analysis of the HIV-1 provirus in chronically infected human T cell lines localized an open chromatin domain downstream of the transcription start site within the Gag "leader" sequence. This region exhibited increased accessibility to DNase I and was designated hypersensitive site 4 (HS 4). Four sequence specific DNA binding factors recognize this region *in vitro*, (*viz.* AP-1, Sp-1, an AP-3 like factor, and a constitutive cellular factor called DBF-1 [Downstream Binding Factor 1]). Point mutations within these sites reduced reporter gene expression in transient transfection assays to 10-20% of the wild-type promoter activity. To address the potential contributions of DBF1 on transcription, oligonucleotides containing this binding site were cloned upstream of HIV-1 TATA box (minimal promoter) linked to a CAT reporter gene. The resulting plasmid constructs were transfected and analyzed using HeLa and Jurkat cell lines. The results showed that DBF1 is a potent transcription activator. Furthermore, IRF1 and IRF2 (a members of the interferon regulatory factor family) are able to bind to a DBF1-site *in vitro* (gel shift assay) and *in vivo* (cotransfection experiments). Cotransfection of IRF1 expressing vector with a construct containing DBF1-sites upstream of the HIV-1 TATA box, resulted in 5 to 10-fold increases in transcriptional activity. (El Kharroubi, Zensen, Martin)

Influence of chromatin structure on HIV-1 transcription regulation. In eukaryotic cells, DNA is organized into nucleosomes and packaged into higher order chromatin fibers. Nucleosome formation may permit or impede the access of soluble transcription factors to their cognate sites in the DNA, thereby playing an active role in transcription regulation. To assess the effect of chromatin structure on HIV-1 promoter, HeLa cells were stably transfected with the wild type HIV-1 LTR linked to the CAT reporter gene. Individual hygromycin-resistant clones were analyzed for expression of CAT activity and the chromatin structure of active and repressed HIV-1 promoters were analyzed by sensitivity to micrococcal nuclease and appropriate restriction enzymes. The results obtained indicate that stably integrated HIV-1 LTR promoter regions are packaged into a distinct nucleoprotein structure which reflects the functional state of the promoter. The active promoter is associated with two nucleosomes separated by a large open chromatin configuration (nuclease hypersensitive [HS]). In an inactive promoter, regulatory sequences upstream of the core promoter (*e.g.* USF, LEF, Ets-1 and NFkB binding sites) are incorporated into an additional nucleosome and the nucleosome, located in the leader sequence, becomes stabilized. These studies provide a physiologically relevant model for studying HIV-1 transcription regulation in the context of chromatin. (El Kharroubi and Martin)

The interaction between HIV-1 Tat and Sp1 modulates phosphorylation of Sp1. We reported previously that HIV-1 Tat binds cellular transcription factor Sp1 *in vivo* and *in vitro*. This finding is consistent with genetic data supporting a critical role for Sp1 in Tat-transactivation of the HIV-LTR. This observation has been extended in two ways. First, the respective domains in Tat and in Sp1 responsible for protein-protein contact has been mapped to Tat amino acids 30 to 55 and Sp1 C-terminal amino acids 531 to 626. Second, we have demonstrated that Tat augments the hyperphosphorylation of Sp1. Since the inherent transcriptional activity of Sp1 is regulated by the extent of its phosphorylation (*i.e.* phosphorylated Sp1 is active; dephosphorylated Sp1 is inactive), this modulatory effect of Tat on Sp1 could represent a critical functional mechanism regulating the transcriptional expression of the HIV-1 LTR. (Chun and Jeang)

Intracellular and biochemical evidence for association of HIV-1 Tat and double stranded RNA-activated protein kinase, PKR. The HIV-1 Tat protein is a vital and potent trans-activator of the HIV-LTR. PKR is an interferon-induced, double-stranded RNA activated protein kinase. It has been reported that PKR activates NF- κ B by phosphorylation of I κ B. Since NF- κ B contributes to Tat-regulated expression of the HIV-1 LTR, we explored whether a functional linkage(s) existed between PKR and Tat. Using GST-fusion protein chromatography of HeLa cellular extracts, amino acids 20 to 72 of Tat were shown to be sufficient for binding to PKR. Additionally, the *in vitro* interaction of Tat with PKR induced novel forms of PKR with reduced mobility in SDS-polyacrylamide gel electrophoresis suggestive of post-translational modification event(s). A portion of the modified PKR was found to be sensitive to serine-threonine phosphatases, a result suggesting that Tat might be able to induce autophosphorylation of PKR and cause its activation. Consistent with this possibility was the observation that over-expression of PKR potently repressed HIV-1 expression/replication. (Chun, Benkirane, Jeang)

Activation of HIV-1 replication in primary monocyte derived macrophage by *Toxoplasma gondii* and soluble *T.gondii* products. Whole, gamma-irradiated *Toxoplasma gondii* and soluble derivatives thereof, have been evaluated for their capacity to activate HIV-1 replication in primary human monocyte-derived macrophage (MDM). The incubation of MDM with *T.gondii* or its soluble products increases both the apparent infectivity of HIV-1 (approx. 50-100 fold) and the amount of progeny virus released into the medium of HIV-1 infected cultures (approx. 5 fold). This effect has been reproduced in eight different donors of MDM. In an initial effort to determine the mechanism of this activation, the release of activating cytokines into the medium of uninfected and HIV-1_{AD8} infected cultures in the presence and absence of *T.gondii* or its soluble products was assayed. Preliminary results indicate that the levels of the primary activating cytokines IL-1, IL-6, and TNF alpha, as well as IFN gamma, in the culture medium are not significantly different from those of control cultures. Treated MDM are also being infected with HIV-1_{AD8} bearing mutations in the NF κ B and Sp1 regions of the viral enhancer in order to begin analyzing possible targets for the *T.gondii* induced activation. (Englund, Martin, Sher, and Gazzinelli)

SYNTHESIS AND PROCESSING OF HIV RNA

The interaction of the HIV-1 Rev protein with its target, the Rev responsive element (RRE). The Rev regulatory protein of HIV Rev is modular protein with a N-terminal RNA binding motif and

near C-terminal activation domain. The N-terminal domain is also required for nuclear localization (NLS) and protein oligomerization. *In vitro* and *in vivo* cross-linking studies have demonstrated that residues between 24 and 35 are involved in Rev oligomerization, an event required for efficient binding to RRE RNA. Four non-overlapping classes of dominant interfering mutants that block the binding of Rev to the RRE were mapped near the N-terminus of Rev. Peptides corresponding to Rev residues between 16 or 23 and 85 and peptides containing poly-arginine residues in place of RNA binding and NLS motifs (35-50) were synthesized. Mutant peptides containing discrete changes in the effector domain, or changes that abolished RRE RNA binding and/or nuclear targeting, and deletion peptides that excised one or both the oligomerization motifs were also synthesized. Wild type Rev peptides corresponding to residues 16/18/23/-85 bound RRE RNA very efficiently; they interacted much less efficiently with TAR, MS2, RexRE RNAs. Smaller peptides with knock-outs in these motifs are being evaluated for their ability to inhibit RNA binding and the cooperativity of wild type Rev peptides. (Krishnan and Venkatesan)

TRBP, a dsRNA binding protein that inhibits interferon regulated PKR kinase, binds to RRE. We have characterized a cellular protein, TRBP that binds to the HIV-1 Rev responsive element (RRE) RNA. TRBP shares significant sequence homology with many cellular and viral ds RNA binding proteins including the interferon inducible ds RNA activated protein kinase, PKR. TRBP is a potent competitive inhibitor of ds RNA activation of interferon induced PKR kinase in vivo and *in vitro*. The yeast two-hybrid system was used to demonstrate dimerization of human double-stranded RNA-dependent protein kinase (PKR) *in vivo* and *in vitro*. TRBP also dimerized with itself and with PKR in the yeast assay. These results suggest that complexes consisting of different combinations of dsRNA-binding proteins may exist *in vivo*. Such complexes could mediate differential effects on gene expression and control of cell growth. (Venkatesan and Sonenberg)

Characterization of cis-inhibitory sequences in HIV-1 gag mRNA. We have identified regulatory sequences within the *gag/pol* gene of HIV-1 which post-transcriptionally repress their own expression, and require the presence of the RRE in cis and expression of *rev* in trans for expression. The determinants of *rev*-dependent and *rev*-independent Gag expression were investigated using the expression plasmid, pCMVgag-2. The presence of a minimal 256 nt RRE in cis (pCMVgag-2RRE) and coexpression of the *rev* in trans (pCMVgag-2RRE/+*rev*) resulted in 10-fold increases in Gag protein expression. Inclusion of the 1.2 kb *env* sequence downstream of the RRE (pCMVgag-2RREL/+*rev*) consistently yielded 100-500 fold increases in Gag expression, indicating that the magnitude of the *rev* response was increased by the addition of *env* sequences other than the RRE. *Rev*-independent increases in *gag* expression of 8-20 fold were achieved by deletion of the HIV-1 major splice donor (MSD), or insertion of the efficiently spliced beta globin IVS-2 intron upstream of the *gag* gene. The combination of MSD deletion + IVS-2 insertion was synergistic, and increased Gag expression 100-200 fold, suggesting at least two independent controls for *Rev*-independent Gag expression. *In situ* hybridization revealed unspliced *gag* RNA expressed from pCMVgag-2, *gag*-2RRE, and *gag*-2RRE/+*rev* accumulated in small granules distinct from the majority of nuclear mRNA. Addition of the beta globin IVS-2 redirected the unspliced *gag* RNA into large clumps which contained the majority of nuclear mRNA and the RNA processing factor SC35. These findings suggest that nuclear export

pathways for *rev*-dependent and *rev*-independent *gag* RNA expression are spatially distinct. (Berthold and Maldarelli)

Localization of HIV-1 RNA transcripts *in situ*. HIV-1 expression proceeds by a regulated program of alternative splicing of full-length 9.2 kb primary transcripts into three size classes of mRNA: 9.2 kb unspliced RNA, 4.5 kb singly spliced RNA, and 1.8 kb multiply spliced RNA. *In situ* hybridization and laser-scanning confocal microscopy revealed two distinct intranuclear locations for the alternatively spliced HIV-1 transcripts. Multiply spliced *tat* RNA accumulated in large clusters containing the non-snRNP RNA processing factor, SC35, while unspliced and singly spliced RNA transcripts were present in smaller granules with little SC35 association. Analyses of recombinant plasmids encoding *gag* alone or *tat* alone revealed expression within the HIV provirus was unnecessary for distinct RNA localization. *gag* RNA localization required specific cis sequences; deletion of an intragenic cis-repressing sequence redirected *gag* RNA from small granules into SC35-containing clusters. Adding the *rev*-responsive element to the deleted construct yielded RNA, which no longer associated with SC35. *tat* RNA localization was a function of a combination of 5' splice site sequences and cis sequences present in the first coding exon of *tat* influence intranuclear RNA localization. (Berthold and Maldarelli)

HIV GAG AND ENV PROTEINS

The function of the HIV-1 matrix and gp41 cytoplasmic tail in envelope incorporation into virions. A proposed role for the matrix (MA) domain of the HIV-1 Gag protein is to facilitate the incorporation of HIV-1 envelope glycoproteins into virus particles. To characterize functions of HIV-1 MA, including envelope incorporation into virus particles, we introduced over 65 single and double amino acid substitution mutations throughout HIV-1 MA. We demonstrated that single amino acid substitutions in MA residues 12 or 30 blocked the virion incorporation of HIV-1 envelope glycoproteins, and that this block could be reversed by pseudotyping with heterologous retroviral envelope glycoproteins containing short cytoplasmic tails, or by truncating 104 or 144 amino acids from the cytoplasmic tail of the HIV-1 transmembrane glycoprotein gp41. To map the domain of the gp41 cytoplasmic tail responsible for the block to virion incorporation imposed by the MA mutations, a series of eight additional truncation mutations were constructed between 23 and 93 amino acids from the C-terminus of gp41. The data indicated that virion incorporation of HIV-1 envelope glycoproteins with truncations of 23, 30, 51, and 56 amino acids from the C-terminus of gp41 is specifically blocked by the MA amino acid 12 mutation, whereas truncations of greater than 93 amino acids reverse the envelope incorporation defect. These results suggest that residues within a predicted α -helix located between 63 and 87 amino acids from the gp41 C-terminus may interact with MA to facilitate the incorporation of envelope glycoproteins with long cytoplasmic tails into HIV-1 virions. To obtain more information about the role of MA and envelope incorporation, we obtained and analyzed viral revertants of two MA residue 12 mutations. Nucleotide sequencing of the revertants indicated that a Val->Ile substitution at MA amino acid 34 compensated for both of the amino acid 12 mutations, providing support for an interaction between residue 12 and 34 during the envelope incorporation process. (Freed and Martin)

Role of the highly basic domain of HIV-1 matrix in macrophage infection. The MA domain of the HIV-1 Gag protein contains a highly-basic region near its amino terminus. It has been proposed that this basic domain, in conjunction with the HIV-1 accessory protein Vpr, is responsible for the translocation of the viral preintegration complex to the nucleus in non-dividing cells. It has also been postulated that the matrix basic domain assists in the targeting of the HIV-1 Gag precursor Pr55^{Gag} to the plasma membrane during virus assembly. To evaluate the role of this highly-basic sequence during infection of primary human monocyte-derived macrophage (MDM), single and double amino acid substitution mutations were introduced, and the effects on virus particle production, Gag protein processing, envelope glycoprotein incorporation into virus particles, and virus infectivity in the CEM(12D-7) T-cell line, peripheral blood mononuclear cells, and primary human MDM were analyzed. Although modest effects on virus particle production were observed with some of the mutants, none abolished infectivity in primary human MDM. In contrast to previously reported studies involving some of same MA basic domain mutants, infectivity in MDM was retained even when combined with a *vpr* mutation. (Freed and Martin)

Molecular genetics of the HIV-1 p6 Gag protein. The HIV-1 Gag protein precursor, Pr55^{Gag}, contains at its C-terminal end a proline-rich, 6 kDa domain designated p6. Two functions have been proposed for p6: incorporation of the HIV-1 accessory protein Vpr into virus particles, and virus particle production. To characterize the role of p6 in the HIV-1 life cycle and to map functional domains within p6, a number of nonsense, single amino acid and multiple amino acid substitution mutations were introduced into p6 and the effects on Gag protein expression and processing, virus particle production, and virus infectivity were analyzed. The production of mutant virus particles was also examined by transmission electron microscopy. The results obtained indicated that: i) p6 is required for efficient virus particle production from a full-length HIV-1 molecular clone; ii) a Pro-Thr-Ala-Pro (PTAP) sequence, located between residues 7 and 10 of p6, is critical for virus particle production; iii) mutations outside the PTAP motif have little or no effect on virus assembly and release; iv) the mutant p6 defect occurs at a late stage in the budding process; and v) mutations in p6 that severely reduced virion production in HeLa cells also block or significantly delay the establishment of a productive infection in the CEM(12D-7) T-cell line. We further demonstrated that mutational inactivation of the HIV-1 protease reversed the p6 defect, suggesting a functional linkage between p6 and the proteolytic processing of the Gag precursor protein during the budding of progeny virions. (Huang, Martin, and Freed)

Inhibition of HIV-1 envelope processing by cyclosporin A. Cyclosporin A is an immunosuppressive drug which has been extensively used in organ transplantation in humans. Recent reports have indicated that treatment of HIV-1 producing cells with cyclosporin A leads to the production of non-infectious virions. To understand the mechanism(s) underlying this phenomenon, the synthesis, processing and release of the HIV-1 structural proteins, transiently expressed from a molecular clone of HIV-1, were biochemically analyzed in a series of pulse/chase analyses. Cyclosporin A treatment severely impaired the processing of the HIV-1 envelope precursor, gp160, leading to a 90 percent reduction in the amount of the mature gp120 and gp41 envelope components incorporated into virions. Biochemical analyses revealed that cyclosporin A reduced the rate of gp160 transport from the endoplasmic reticulum, altered processing of asparagine-linked carbohydrate associated with gp160 in the Golgi complex, and impaired the proteolytic cleavage of gp160 to gp120 and gp41. Two non-immunosuppressive analogs of cyclosporin A are under investigation in HIV-1 infected PBMC and monocyte-derived-macrophage systems. (Willey, Freed, Maldarelli and Martin)

Differential glycosylation and sensitivity to neutralizing antibodies of HIV-1 envelope produced from primary T-cell and macrophage cultures. Two primary targets of HIV-1 infection *in vivo* are CD4⁺ T-cells and monocyte-derived-macrophages (MDM). SDS/PAGE analyses of the virion-associated envelope proteins and particles released from infected peripheral blood mononuclear cells (PBMCs) and MDM indicated that HIV-1 envelope proteins produced each are biochemically distinct. The MDM-derived gp120 migrated as a broadly diffuse band compared to the more homogeneous gp120 released from PBM. Treatment of the different envelope populations with the glycosidase PNGaseF, demonstrated that the mobilities of the two gp120s were due to differences in asparagine-linked glycosylation. Although infectivity studies revealed that virus produced from PBMC or MDM was not significantly altered in its ability to establish a productive infection in either cell type, neutralization experiments carried out with human PBMC indicated that the MDM-derived HIV-1 required up to seven-fold more neutralizing antibody to completely block infection. These results suggest that the cell of origin not only affects the glycosylation of HIV-1 gp120, but can alter the susceptibility of the resultant viral progeny to neutralization. (Willey, Shibata and Martin)

INTEGRASE

A defective U5 LTR deletion mutant of HIV-1 reverts by eliminating an additional 19 nucleotides from its genome. Non-overlapping deletions that eliminated the 5' (HIV-1_{U5/603del}), middle (HIV-1_{U5/206del}) and 3' (HIV-1_{U5/604del}) thirds of the U5 region of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) were studied for their effects on virus replication (transient transfection of HeLa cells) and infectivity (T cell lines and PBMCs). All three mutants exhibited a wild type phenotype in directing the production and release of virus particles from transfected HeLa cells. In infectivity assays, HIV-1_{U5/206del} was usually indistinguishable from wild type virus whereas HIV-1_{U5/603del} was unable to infect human PBMCs or MT4 and CEM cells. Investigations of HIV-1_{U5/603del} particles revealed a packaging defect resulting in a 10-fold reduction of encapsidated genomic RNA. The HIV-1_{U5/604del} mutant was either noninfectious or exhibited delayed infection kinetics, depending on the cell type and multiplicity of infection. Quantitative competitive PCR indicated that HIV-1_{U5/604del} synthesized normal amounts of viral DNA in newly infected cells. During the course of a long term infectivity assay, a revertant of the HIV-1_{U5/604del} mutant emerged that displayed rapid infection kinetics. Nucleotide sequence analysis indicated that the original 26 nucleotide (nt) deletion present in HIV-1_{U5/604del} had been extended an additional 19 nts in the revertant virus. Characterization of the HIV-1_{U5/604del} mutant LTR in *in vitro* integration reactions revealed defective 3' processing and strand transfer activities that were partially restored when the revertant LTR substrate was used, suggesting that the reversion corrected a similar defect in the mutant virus. (Vicenzi, Purcell, Englund, Dimitrov, and Martin)

The integration of HIV DNA is required for productive infection of primary human macrophage. Certain human immunodeficiency virus type 1 (HIV-1) isolates are able to productively infect nondividing cells of the monocyte/macrophage lineage, such as microglial cells in the central nervous system of seropositive individuals. A molecular genetic approach has been used to construct two different *IN* gene mutants of HIV-1 that were studied in the context of an infectious macrophage tropic HIV-1 molecular clone. One mutant, HIV-1_{ΔD(35)E}, containing a 111 bp deletion that eliminated the central catalytic domain of the IN protein, was noninfectious in

both peripheral blood mononuclear cells (PBMC) and monocyte derived macrophages (MDM). The HIV-1_{ΔD35E} mutant, however, exhibited defects in both assembly/release of progeny virions and viral DNA synthesis during the early stages of a *de novo* MDM infection compared to wild type virus. The second mutant, HIV-1_{NL(ΔD8)(D116N/6)}, containing a single aspartic acid to alanine substitution at the invariant Asp₁₁₆ residue of IN, was also noninfectious in both PBMC and MDM but, in contrast to the IN deletion mutant, was indistinguishable from wild type virus in particle production. PCR analysis indicated that the HIV-1_{NL(ΔD8)(D116N/6)} IN mutant entered MDM, reverse transcribed its RNA, but was unable to complete its replication cycle because of a presumed block to integration. These data establish integration as an obligate step in HIV-1 infections of activated PBMC and primary human macrophage cultures by cell free virus preparations. (Englund, Theodore, Freed, and Martin)

Delineation of att sites at the termini of HIV DNA recognized by IN. Previous studies of a deletion mutant of HIV-1_{NLΔ-3} lacking 26 nucleotides from the U5 region (HIV-1_{604del}) indicated that this sequence was required for efficient integration both *in vitro* and *in vivo*. A revertant of 604del, HIV-1_{revEI}, which replicated with near wildtype kinetics and integrated more efficiently *in vitro* was isolated, and was found to have extended the original deletion by an additional 19 nucleotides in the 5' direction. Using oligonucleotides corresponding to the U5 termini of the wildtype, mutant, and revertant proviruses, a study has been initiated to identify DNA sequences/structures required for efficient integration. Using mutant oligonucleotides whose sequences are intermediate between wildtype and revertant, at least two (T @ -7, and A @ -2, where the C in the absolutely conserved CAGT motif at the 3' end of U5 is position 1) residues have been identified which seem critical for enhanced integration efficiency *in vitro*. In addition, NMR studies of potentially significant oligonucleotides are being conducted. (Englund, Martin, and Dimitrov)

HIV VPU, VIF, AND NEF

Structural and functional analysis of the HIV-1 Vpu gene product: the two functions of Vpu are regulated by separable structural domains. Vpu is an integral membrane protein encoded by HIV-1. Vpu regulates the secretion of progeny virions from infected cells but also causes degradation of the virus receptor CD4 in the endoplasmic reticulum (ER). These two Vpu functions are independent of one another. We have previously reported that sequences in the cytoplasmic domain of Vpu mediate CD4 degradation and have shown that the transmembrane (TM) domain of Vpu functions merely as a membrane anchor which can be substituted by heterologous TM domains without impairing the capacity of Vpu to induce CD4 degradation. Our data also suggests that the TM domain of Vpu plays a critical role in regulating virus particle release. Randomization of the TM sequence did not affect membrane integration or the topology of Vpu and the resulting protein was capable of inducing CD4 decay. However, this latter mutant Vpu protein failed to mediate virus release. Preliminary studies suggest that the TM domain of wild type Vpu can form an ion channel while no such activity was detected for the randomized TM domain. Experiments are in progress to further characterize the possible ion channel activity of Vpu. (Schubert, Strebel)

Mechanism of CD4 degradation by Vpu: the Vpu responsive sequence in CD4. CD4 is an integral membrane glycoprotein which functions as the HIV receptor for infection of human host cells. We have recently demonstrated that Vpu induces rapid degradation of CD4 in the

endoplasmic reticulum. Using an *in vitro* system, it was also shown that Vpu targets specific sequences in the cytoplasmic domain of CD4 during the degradation reaction. Transfer of CD4 cytoplasmic sequences to heterologous proteins resulted in Vpu-mediated degradation of the resultant chimeric proteins. Further analysis of the molecular mechanism demonstrated that CD4 degradation involves a direct protein-protein interaction between Vpu and CD4. Several biologically inactive Vpu mutants were still able to bind to CD4 but did not cause CD4 decay. We conclude that binding of Vpu to CD4 is a necessary but insufficient step in Vpu induced CD4 degradation. Our data suggest that CD4 degradation is a multi-step process which is initiated by binding of Vpu to its target but which requires additional catalytic activities of Vpu. (Bour, Strebel)

The mechanism of Vpu-mediated particle release. We previously reported that Vpu-mediated degradation of CD4 requires the participation of sequences in the cytoplasmic domains of both Vpu and CD4. To analyze whether Vpu-mediated enhancement of virus release is similarly specific, the effects of Vpu on HIV-2 were investigated. HIV-2, unlike HIV-1, does not encode a Vpu protein or a known functional homologue. Analysis of virus release kinetics from transfected HeLa cells indicated that HIV-2 virus release, in the absence of Vpu, is extremely efficient and comparable to that of HIV-1 in the presence of vpu. However, when HIV-2 gag/pol genes were expressed in the context of the HIV-1 backbone, particle release was dramatically reduced in the absence of Vpu; co-expression of Vpu with the HIV-1/HIV-2 chimera resulted in increased virion release. To further investigate this phenomenon, plasmids were constructed that express various portions of the HIV-2 genome. Cotransfection of such constructs with the HIV-1/HIV-2 chimera expressing HIV-2 gag/pol, in the context of the HIV-1 backbone, indicated that sequences located in the 3'-half of the HIV-2 genome enhanced the release of HIV-2 particles. This result suggests that HIV-2 encodes a factor which, like Vpu, is capable of enhancing release of virions. Studies are ongoing to identify and characterize this previously unreported HIV-2 factor. (Bour, Strebel)

Down-regulation of cell surface CD4 by HIV-1 *rev*, *vpu* and *env*. Studies with transient expression systems have shown that HIV Env and Vpu are involved in down-regulating CD4, the HIV receptor. To study the effects of these proteins in a stable expression system, a retroviral vector containing HIV-1 *rev*, *vpu* and *env* was constructed and introduced into HeLa-CD4 cells by retroviral-mediated gene transfer. Cell surface CD4 was decreased more than 10-fold in some cells transduced by this vector, but not in cells transduced with the same vector containing mutations in either *vpu* or *env*. A novel feature of the stable expression system is that individual clones of transduced cells consisted of two distinct populations of cells: one with greater than 10-fold reduced surface CD4 and one with parental levels of CD4. When the two cell populations were separated by FACS, those with high levels of surface CD4 gave rise to cells with low surface expression of CD4 and vice versa after several days in culture. There was an inverse correlation between levels of surface CD4 and intracellular Vpu, and cells with high Vpu had noticeable concentration of Vpu in the perinuclear region. The bi-modal distribution of surface CD4 observed appears to result from accelerated degradation of CD4 and /or reduced transport to the cell surface in cells containing greater than a threshold amount of Vpu. The apparent threshold effect of Vpu on CD4 is consistent with a model in which the active form of Vpu is a multimer or resides in a post-endoplasmic reticulum compartment of the cell. This model describing the bi-modal distribution of CD4, could provide insight into the function of other cell proteins whose expression is also bi-modal in single populations of cells. (Fujita, Silver)

Subcellular location of the Vif protein. Vif is a 23 kDa basic protein encoded by all primate lentiviruses. Even though the function of Vif has not yet been resolved, it is apparent from numerous biological studies that the protein has an important role in regulating virus infectivity. Cell fractionation and confocal microscopic studies indicate that Vif associates with the cytoskeleton, specifically the intermediate filament (IF) system. Approximately 40 to 50% of intracellular Vif is associated with the cytoskeleton and, in contrast to a published report, only a minor fraction of Vif appears to be membrane-bound. Association of Vif with IFs is specific and can result in the reorganization of the intermediate filaments. Ongoing experiments are designed to determine the significance of the cytoskeletal association of Vif during different phases of the virus life cycle. (Karczewski, Strebel)

The cellular effects of the HIV-1 Nef protein. Among the accessory proteins encoded by HIV-1, the 27 kD myristoylated Nef protein has been implicated in down-regulation of the CD4 and IL-2 receptors, IL-2, and enhancer binding proteins, NF κ B and AP-1. Nef induced CD4 downregulation is bi-modal. When Nef is acutely expressed in CD4 + cell lines, the predominant effect is rapid endocytosis and degradation of CD4 at the cell surface. A 20 amino-acid cytoplasmic tail sequence of CD4 is necessary and sufficient for this effect. However, when Nef is acutely co-expressed with CD4 in non-lymphoid cells or CD4 negative T cells, it interferes with CD4 biosynthesis. The direct physical interaction of Nef with the β -COP coatomer subunit component of non-clathrin-coated vesicles has been recently reported. β -COP is an essential component of the non-selective vesicular ER to the Golgi transport system. Nef may delay the acquisition of nascent CD4 by the coatomer vesicles and thereby induce CD4 proteolysis. β -COP has significant homology to the β -adaptin component of clathrin-coated vesicles. It is conceivable that Nef may potentially interact with the β -adaptins as well. By virtue of interaction with the key components of coated vesicles, Nef may modulate both the antero-grade transport and endocytosis of other cellular proteins as well. (Chandrasekhar, Popov and Venkatesan)

HIV Nef protein interacts with cellular proteins. The requirement of a competent Nef gene for the maintenance of virus load in the SIV_{mac}/rhesus monkey system suggests that Nef may positively affect virus replication *in vivo* by inducing T cell activation. A recent report showed that Nef may activate or inhibit signalling events from the T cell receptor depending on subcellular localization. Many of these divergent observations may reflect the ability of Nef to modulate cell physiology by modulating complex intracellular signalling pathways. Preliminary reports have suggested that sub-domains of Nef may interact with the SH3 (*src* homology 3) domains of *src* related oncogenes. Using the yeast two hybrid system to screen a cDNA library of HeLa cells for proteins that interact with HIV Nef, two cDNA clones (RK29 and RK50) were obtained. RK 29 encodes a polypeptide of 255 amino acids. RK29 is a nuclear protein that associates with the unmyristoylated Nef protein in HeLa cells. The acidic C-terminal portion of RK29 shares homology with eukaryotic transcription factors. (Krishnan and Venkatesan)

HIV DISEASE AND VACCINE MODELS

Isolation and characterization of a syncytium-inducing, macrophage/T-cell line tropic HIV-1 isolate that readily infects chimpanzee cells *in vitro* and *in vivo*. Fresh HIV-1 isolates from AIDS patients were screened for infectivity in chimpanzee peripheral blood mononuclear cells (PBMC) to identify strains potentially able to generate high virus loads in an inoculated animal. Only 3 of 23 isolates obtained were infectious in chimpanzee cells. Of these 3, only one (HIV-1_{DH12})

was able to initiate a productive infection in PBMC samples from all 25 chimpanzees tested. HIV-1_{DH12} tissue culture infections were characterized by extremely rapid replication kinetics, profound cytopathicity, and tropism for chimp and human PBMC, primary human macrophage, and several human T cell lines. An infection was established within one week of inoculating a chimpanzee with 50 TCID₅₀ HIV-1_{DH12}; cell free virus was recovered from the plasma on weeks 1, 2, and 4 and was associated with the development of lymphadenopathy. Virus loads during the primary infection and at 6 months post-inoculation were comparable to those reported in HIV-1 seropositive individuals. (Shibata and Martin)

Molecular characterization of HIV-1 isolates capable of infecting chimpanzee PBMCs. In an attempt to identify viral determinants responsible for the chimpanzee tropism and the virulence factors associated with HIV-1_{DH12}, primary isolate, 12 chimeric viruses were generated containing either the entire envelope (gp120 and gp41, or individual and combinations of the variable regions (V1-5) of HIV-1_{DH12}, introduced into the backbone of another HIV-1 isolate (HIV-1_{AD8}), which is unable to infect chimpanzee PBMCs and human T-cell lines (AD8DHenv, AD8DH120, AD8DH41 and AD8DH120A-I, respectively). The chimeric viruses AD8DHenv and AD8DH120, but not AD8DH41, were able to infect chimpanzee PBMCs. In addition, the replication kinetics of AD8DHenv and AD8DH120 in human PBMCs were similar to that of HIV-1_{DH12}, and significantly faster than that of HIV-1_{AD8}. The chimeric virus containing all 5 variable regions of gp120 (AD8DH120I), but not those which only contain individual variable domains, was also infectious for chimpanzee PBMCs and replicated rapidly in human PBMCs. Interestingly, an analysis of the infectivity of the chimeric viruses in the human MT4 cell line revealed that the ability to replicate in T-cell lines correlated with tropism for chimpanzee PBMCs. These results indicate that gp120 contains determinants which confer chimpanzee tropism in HIV-1 and infectivity in chimpanzee cells and T-cell lines requires the cooperative interaction between all of the variable regions. (Cho, Shibata, Martin)

Simultaneous inoculation of a chimpanzee with 3 different HIV-1 primary isolates. A chimpanzee was simultaneously inoculated with large amounts of three different primary HIV-1 isolates (HIV-1_{DH12}, HIV-1_{DH20}, and HIV-1_{DH29}) as both cell-free and cell-associated virus. During the primary infection, HIV-1_{DH12} was isolated directly from the plasma and HIV-1_{DH20} DNA was detected following amplification of PBMC DNA between weeks 4 and 11. Following resolution of the initial infection, the immune system of the infected chimpanzee was stimulated by two infusions of human PBMCs at weeks 23 and 28 post inoculation. This resulted in the appearance of HIV-1_{DH29} in lymphocytes recovered from a lymph node biopsy. The subsequent administration of high doses of steroids induced a plasma viremia at weeks 48 and 70, marked elevations of ELISA antibody levels, and increased numbers of PBMC carrying copies of HIV-1 DNA. (Shibata and Martin)

Development of a SHIV/Macaque monkey disease model. Seven cynomolgus macaques, one rhesus macaque, and two pig-tailed macaques have been inoculated with the HIV-1/SIV chimeric virus (SHIV) that carries the *vpr-tar-rev-vpu-env-nef* gene region from the HIV-1_{DH12} isolate (and designated SHIVmad1). All of the monkeys became infected. The animals are being monitored for clinical symptoms, alterations of lymphocyte subsets, SHIV virus load, and antibody response. One of cynomolgus monkeys exhibited significant drop of CD4+ cells (less than 100 cells/ μ l blood) which has persisted for more than a year. This monkey has recently developed a wasting syndrome beginning approximately 60 weeks after inoculation, which has resulted in a loss of more than 30% body weight. Some of the other monkeys have exhibited a transient drop of CD4

cells, and one of the pig-tailed macaques has experienced a gradual decrease of CD4 positive lymphocytes (16 weeks post inoculation). Two cynomolgus macaques have recently been transfused with blood from the animal with low CD4 cell levels. Since the SHIVs isolated from several of the infected monkeys replicate more rapidly and to higher titers than the original virus, several molecular clones corresponding to these viruses have been obtained and are being evaluated for infectivity in both tissue culture systems and *in vivo*. Some of the persistently infected macaques with relatively low virus load and no CD4 depletion are being treated with immunosuppressive agents in an attempt to increase levels of circulating SHIV. (Shibata, Matano, Maldarelli, Theodore, Siemon, and Martin)

Superchallenge of previously HIV-1 infected, asymptomatic chimpanzees with a second HIV strain (a model of an HIV-1 attenuated vaccine). Because HIV-1_{IMB} or HIV-1_{SF2} fails to induce disease in chimpanzees, animals infected with these 2 isolates can be viewed as having been vaccinated with an attenuated viral vaccine. One naive chimpanzee, two HIV-1_{IMB}-infected chimpanzees, and two HIV-1_{SF2}-infected chimpanzees were challenged with HIV-1_{DH12} (a human/chimpanzee tropic strain of HIV-1 recently isolated from an AIDS patient). The naive animal, which was inoculated with 50 TCID₅₀ of HIV-1_{DH12}, became infected within 1 week of challenge as monitored by viral RNA in the plasma, viral DNA in PBMC or in lymph node cells, and HIV-1_{DH12}-specific neutralizing antibody. The "vaccinated animals" exhibited various levels of protection against HIV-1_{DH12} (none of them exhibited plasma viremia). One HIV-1_{IMB}-infected animal was resistant to successive challenges with 170 and 1700 TCID₅₀ of HIV-1_{DH12}. HIV-1_{DH12} was never detected in the PBMC or lymph nodes by nested DNA PCR. This animal did develop neutralizing antibody against HIV-1_{DH12} following a blood transfusion from an HIV-1_{DH12}-infected animal (the blood contained approximately 300 infected cells) but no HIV-1_{DH12} was detected by PCR. This result suggests that a low level HIV-1_{DH12} infection had probably occurred. The other HIV-1_{IMB}-infected animal was challenged with 1700 TCID₅₀ of HIV-1_{DH12}, and developed neutralizing antibody against HIV-1_{DH12}, but HIV-1_{DH12} was not detected by PCR, even after a rechallenge with 17000 TCID₅₀. Although HIV-1_{DH12} could be detected by DNA PCR from both HIV-1_{SF2} previously infected animals after a challenge with 120 TCID₅₀ of HIV-1_{DH12}, the amount of PBMC-associated viral DNA was reduced by more than 30 fold than the control animal. None of the four "vaccinated" animals had neutralizing antibody against HIV-1_{DH12} prior to the challenges with HIV-1_{DH12} (but had neutralizing antibody against the pre-existing virus stains [HIV-1_{IMB} or HIV-1_{SF2}], suggesting that a cellular rather than a humoral immune mechanism was responsible for the resistance to HIV-1_{DH12} observed. CTL assays are in progress using PBMC from these animals. (Shibata, Siemon, Cho, and Martin).

Analysis of the cellular immune response to HIV-1 in chimpanzees. Although HIV-1_{DH12} readily induces a viremia following infection of naive chimpanzees, animals previously infected with HIV-1_{IMB} and HIV-1_{SF2} are resistant to HIV-1_{DH12} challenge. Because neutralizing antibodies against HIV-1_{DH12} were not detected in the HIV-1_{IMB} and HIV-1_{SF2} infected animals, it is very likely that cellular immune responses are responsible for preventing that chimpanzees from becoming infected with HIV-1_{DH12}. To examine this possibility, PBMC from the HIV-1_{IMB} and HIV-1_{SF2} infected chimpanzees are being analyzed for cytotoxic T-lymphocyte activity directed against various HIV-1 isolates. B-lymphoblastoid cell lines (BLCL) from each of these chimpanzees have been established and a recombinant vaccinia virus (vVDHenv) which expresses the Env protein of DH12 strain has been constructed. Using BLCL infected with vVDHenv as antigen presenting cells, frozen chimpanzee PBMC, collected at various times prior to and following infection with HIV-1_{DH12} are currently being screened. (Cho, Shibata, Martin)

HIV-1 that expresses HSV-1 thymidine kinase. Attenuated viruses are promising reagents for HIV vaccines. The current approach for making an attenuated live HIV uses a loss-of-function strategy (*i.e.* deletion of the accessory *nef* gene). We propose that a gain-of-function approach could also create attenuated live viruses. As an example, an HIV-1 that expresses the HSV-1 thymidine kinase gene has been constructed. In principle, production of HSV-1 thymidine kinase (TK) would confer ganciclovir (GCV) sensitivity to this chimeric virus. This recombinant HIV-TK virus has a pNL4-3 backbone with the first 80 bases of *nef* removed. In a thymidine kinase assay using LTK⁻ cells, HIV-TK produced 3.6 fold more activity than pHSV-106 (a plasmid containing the HSV-1 TK gene), while pNL4-3, as expected, showed no enzymatic activity. We found that GCV treatment of infected CEM cells eliminated the replication of HIV-TK while the parental NL4-3 virus was unaffected by drug. To prove that GCV treatment completely eradicated virus, PCR analysis of infected cell genomic DNA was performed. In the GCV-treated HIV-TK cultures, no viral DNA was detected, while control infections yielded the expected bands. Although additional work is necessary to optimize development of this approach, the current findings encourage further investigation of a gain-of-function strategy for attenuated vaccine development. (Smith and Jeang)

HTLV I TAX

A novel b-ZIP protein binds HTLV-I Tax. The Tax protein, encoded by human T-cell leukemia virus type I (HTLV-1), is a potent transcriptional activator of viral and cellular genes. Tax does not bind DNA directly but may interact with host cell transcription factors that do recognize the HTLV-I LTR. A cDNA that codes a Tax-binding protein was isolated from a human library using the yeast two hybrid approach. This cDNA has been sequenced and has no known counterpart in existing databases. Its deduced amino acid sequence encodes a 48kDa protein containing an intact leucine zipper motif flanked in its N-terminal direction by a basic region. Hence, this novel protein fulfills one definition for the b-ZIP family of regulatory factors. The minimally sufficient sequence for Tax-binding and for self-multimerization was localized to a predicted helical region immediately upstream of the leucine zipper. This novel b-ZIP protein, provisionally designated as TBF18-1, when fused to the DNA binding domain of Gal4, activates a minimal promoter in yeast and in mammalian cells. These findings suggest that TBF18-1 is a Tax-binding factor that might have a co-activator function. (Jin and Jeang)

Subnuclear localization of Tax in "speckled" structures. Proteins have been subdivided generally into nuclear and cytoplasmic moieties. While it is clear that nuclear localization is important for HTLV-I Tax function, it is unknown whether there are additional subnuclear determinants of activity. Using confocal microscopy we have defined the subnuclear address for Tax. Inside the nucleus, Tax forms discrete "speckled" structures (TSS). TSS were found in Tax-transfected as well as HTLV-I transformed cells. They are congruent with a previously described "spliceosome" pattern and co-localization was verified by double-staining with a "spliceosome"-specific monoclonal antibody. Furthermore, the physical association of Tax with "spliceosomes" was documented by co-immunoprecipitation from cell-free extracts. The functional importance of this subnuclear localization was supported by the failure of transcriptionally inactive Tax mutants, which were competent for nuclear localization, to target to the TSS. Additional characterization of TSS revealed that they overlap with a subset of "hot-spots" for transcription, which stained immunologically for RNA Pol II, TBP and CREB. In several experiments, "heat-

shock" treatment disrupted the subnuclear Tax-structure and abolished transcriptional activity. (Semmes and Jeang)

MURINE RETROVIRUSES

Mouse chromosomal genes responsible for resistance to murine leukemia viruses. A number of mouse genes have been identified which control susceptibility to specific murine retroviruses and the diseases they induce. During the process of analyzing genetic crosses segregating the previously described resistance gene, *Rmcf*, three novel genetic factors were identified in *M. castaneus* parental mice, which interact to produce resistance to the mink cell focus forming subgroup of mouse leukemia viruses. One of these genes was mapped to the Chromosome 1 region containing the cell surface receptor for this virus suggesting that *M. castaneus* may have an allelic variant of the receptor. The other two genes in these mice may encode viral envelope glycoproteins which prevent the receptor from binding exogenous virus. (Kozak and Lyu)

Envelope-mediated resistance to retroviruses. A strain of transgenic mice that was resistant to infection with ecotropic murine leukemia virus because of inheritance of a highly expressed retroviral *env* gene (*Fv4*) was recently constructed. Serum from these mice were analyzed to ascertain whether it contained a factor capable of blocking infection. Ecotropic virus titers were reduced about 100-fold when titered in NIH3T3 cells that were pre-incubated with transgenic mouse serum instead of control mouse serum or no serum. *Fv4* serum contained a factor, presumably encoded by the transgene, that bound to cells expressing the ecotropic virus receptor and reacted with monoclonal antibody to the *Fv4* Env protein. Tail cells from transgenic mice and NIH3T3 cells transduced with the transgene synthesized the *Fv4* Env as a gp85 Env precursor. This precursor was processed to gp70 and p15E moieties, and the gp70 species was secreted as a free (non-particle-associated) protein. Studies with bone marrow chimeras showed that both at hemopoietic and at non-hemopoietic cells contributed to serum *Fv4* Env. These experiments indicate that secreted Env protein can mediate resistance to retroviral infection and play an important role in natural retroviral resistance mechanisms. (Nihrane, Silver)

An array of MuLV-related elements are transmitted and expressed in a primate recipient of retroviral gene therapy. Direct RNA-PCR analyses of T-cell lymphomas that developed in rhesus macaques during a gene therapy experiment revealed the presence of several different recombinant murine leukemia viruses (MuLV). Most prominent was the expected MuLV recombinant, designated Mo_{LTR}Ampho_{env}, in which the amphotropic *env* of the helper packaging virus was joined to the long terminal repeat (LTR) of the Moloney (Mo) MuLV derived vector. This retrovirus does not exist in nature. An additional copy of the core enhancer acquired from the vector LTR greatly augmented the growth potential of Mo_{LTR}Ampho_{env} MuLV in several different rhesus cell types compared to prototypical amphotropic MuLV_{4070A}. Unexpectedly, at least two types of mink cell focus forming (MCF) MuLV elements, arising from endogenous retroviral sequences expressed in the murine packaging cell line, were also transmitted and highly expressed in one of the macaques. Furthermore, murine leukemia virus-like VL-30 sequences were detected in one rhesus lymphoma, but these were not transcribed into RNA. The unanticipated presence of an array of MuLV-related structures in a primate gene therapy recipient demands ever-vigilant scrutiny for the existence of transmittable retroviral elements and replication competent viruses possessing altered tropic or growth properties in packaging cells producing retroviral vectors. (Purcell and Martin)

MAPPING MOUSE GENES

Genetic linkage studies in the mouse. Analysis of somatic cell hybrids and two sets of inter(sub)species crosses have been used to develop a genome-wide map of the mouse which, at present, contains over 750 markers. This map consists largely of expressed genes and their pseudogenes. Some of the most recently mapped genes include a brain cDNA, sulfotransferases, novel protein tyrosine kinases, opioid receptors, and prosaposin. One cartilage-derived morphogenetic factor was identified as a candidate for the skeletal mutation, brachypodism. Acquisition of a panel of human x rodent somatic cell hybrids has also made it possible to define the chromosomal assignments of the human homologs of some of these genes. (Kozak, Adamson, Filie, Lyu)

EUCARYOTIC RETROTRANSPOSITION

RNA-mediated recombination in eukaryotic cells. A yeast model system has been developed that detects the reverse transcription of cellular RNA and the chromosomal insertion of the cDNA transcript (i.e., RNA-mediated recombination). The yeast LTR-containing retrotransposon, Ty, provides the source of reverse transcriptase and is also required for priming reverse transcription of the cellular transcript. Priming by Ty occurs via a template switch: reverse transcription is initiated on the Ty transcript and then switches onto the poly(A) tail of the cellular transcript. A LINE-like element, CRE, from the trypanosome *Crithidia fasciculata*, has also been tested for its ability to reverse transcribe cellular transcripts. Because CRE is a non-LTR containing element or poly(A) retrotransposon, its mechanism of priming and reverse transcription (unknown at present) must be different from Ty. It has been suggested that priming by LINE elements may initiate from a nick in the chromosome. Therefore, the yeast topoisomerase I gene has been overexpressed to increase chromosomal nicks. This has resulted in an increase in cDNA insertions. These insertions are currently being analyzed to learn more about the priming mechanism used by LINE elements. Insertion of the cDNA into the chromosome can also be mediated by the cellular recombination machinery. An assay has been developed to specifically identify the cellular genes required for recombination between a diffusible cDNA and its chromosomal allele. Using this assay, we have found that the RAD52 (involved in most recombination in yeast) and RAD1 (involved in excision repair in yeast) gene products are required for RNA-mediated gene conversion of a chromosomal allele. (Derr)

SEMLIKI VIRUS VECTORS

Semliki Forest Virus (SFV) expression system for retroviral genes. A significant problem with retroviral-mediated gene transfer systems is that retroviral particles and proteins are expressed at fairly low levels compared to other viral gene expression systems. A gene expression system based on Semliki Forest Virus has recently been developed that produces very high levels of vector RNA and, in some cases, of vector-encoded proteins. We used this system to express retroviral *gag-pol* and *env* and have developed SFV vectors that express functional retroviral Gag and Env proteins and very high levels of retroviral-related RNAs. This hybrid SFV-retroviral

system has potential uses for vaccination against retroviruses as well as for retroviral-mediated gene transfer. (Lebedeva, Silver)

PATHOGENIC MYCOPLASMA

Mycoplasma Occurrence and Pathogenicity. In a patient dually infected with M. pneumoniae and M. genitalium, an immune response was demonstrated to several mammalian cytoskeletal components (keratin, myosin, and fibrinogen), suggesting that sequence homology between mycoplasmal adhesins and human tissue may play an important role in host autoimmune responses and mycoplasmal pathogenicity. We have also identified twenty-four isolates of M. pneumoniae in the genital tract of twenty-two females attending several gynecologic clinics. An explanation for this unusual occurrence and the potential role of the organism in various clinical conditions noted are under investigation. (Tully)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00027-28 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others: None

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1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current efforts emphasize the biology and pathogenicity of Mycoplasmas of human origin and, where important, other mycoplasmas from animal hosts. Work continues on the putative role of Mycoplasma genitalium in human arthritic infections, extending our earlier report on the occurrence of a mixed infection with this organism and M. pneumoniae in synovial tissues of an immunologically competent patient. Although a dual infection was clearly documented, we could not identify either mycoplasma as a dominant etiologic agent of the observed disease. We have since demonstrated that during the mixed infection, the patient developed an immune response to several mammalian cytoskeletal proteins, findings that offer further support to the concept of a postinfectious autoimmune mechanism in mycoplasmal diseases. In further searches for M. genitalium, we identified twenty-four M. pneumoniae isolates in lower genital tract specimens from females attending a number of different gynecologic clinics. In at least one instance, the organism was also isolated from the urethra of a male cohort of one infected female. Since a number of the patients admitted to oral/genital contact, this activity is probably the most likely means of acquisition and/or transmission of the mycoplasma. This is the first extensive documentation of this organism at this anatomical site. Mycoplasma hominis infections were identified in two lung transplant patients, with donor tissue as the most likely source of infection. These clinical mycoplasmal infections continue to raise important management questions, since prolonged immunosuppressive therapy and the ineffectiveness of antibiotics in the absence of a functioning immune system prolongs and complicates recovery. Also, we recently identified a M. felis infection in the joint tissues of a hypogammaglobulemic patient, an organism not previously reported in man. The widespread occurrence of mycoplasmas in animals and their ability to colonize human mucosal or synovial tissues have important consequences for the diagnostic laboratory and for clinical medicine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00190-17 LMM

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October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Genetics of Eukaryotic Cells and Their Viruses

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PROFESSIONAL:

4

OTHER:

1

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A proposed role for the matrix (MA) domain of the HIV-1 Gag protein is to facilitate the incorporation of HIV-1 envelope glycoproteins into virus particles. To characterize functions of HIV-1 MA, including envelope incorporation into virus particles, we introduced over 65 single and double amino acid substitution mutations throughout HIV-1 MA. We demonstrated that single amino acid substitutions in MA residues 12 or 30 blocked the virion incorporation of HIV-1 envelope glycoproteins, and that this block could be reversed by pseudotyping with heterologous retroviral envelope glycoproteins containing short cytoplasmic tails, or by truncating 104 or 144 amino acids from the cytoplasmic tail of the HIV-1 transmembrane glycoprotein gp41. To map the domain of the gp41 cytoplasmic tail responsible for the block to virion incorporation imposed by the MA mutations, a series of eight additional truncation mutations were constructed between 23 and 93 amino acids from the C-terminus of gp41. The data indicated that virion incorporation of HIV-1 envelope glycoproteins with truncations of 23, 30, 51, and 56 amino acids from the C-terminus of gp41 is specifically blocked by the MA amino acid 12 mutation, whereas truncations of greater than 93 amino acids reverse the envelope incorporation defect. These results suggest that residues within a predicted α -helix located between 63 and 87 amino acids from the gp41 C-terminus may interact with MA to facilitate the incorporation of envelope glycoproteins with long cytoplasmic tails into HIV-1 virions. To obtain more information about the role of MA and envelope incorporation, we obtained and analyzed viral revertants of two MA residue 12 mutations. Nucleotide sequencing of the revertants indicated that a Val->Ile substitution at MA amino acid 34 compensated for both of the amino acid 12 mutations, providing support for an interaction between residue 12 and 34 during the envelope incorporation process.

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PROJECT NUMBER

Z01 AI 00300-14 LMM

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October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic aspects of viral oncogenesis in wild mouse species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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1.5

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1.0

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0.5

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- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Many mouse chromosomal genes are known which affect the susceptibility of mice to retrovirus-induced neoplastic disease. These genes include endogenous retroviral sequences, mouse cellular genes which facilitate or restrict virus replication, and proto-oncogenes disrupted by viral insertion. We have been using standard classical genetics to identify and map genes involved in these phenomena. In one series of experiments, we obtained a molecular clone of the receptor for the amphotropic MuLV cell surface receptor, and we defined the map location of this gene in the mouse. We have also mapped TRBP, a positive regulator of HIV-1, in man and mouse. In other experiments, we have continued analysis of several crosses involving DBA/2J mice which carry a locus, *Rmcf*, responsible for resistance to the mink cell focus inducing (MCF) subtype of mouse leukemia viruses. We defined the genetic map location of *Rmcf*; we identified an additional resistance gene in the *M. castaneus* parent of these same crosses which maps to Chr 1; we identified two additional factors which contribute to this resistance in *M. castaneus*; and we are following inheritance of *Rmcf* in serial backcrosses to *M. castaneus* which lacks endogenous MCF viruses. These experiments should help determine whether *Rmcf* represents an endogenous provirus, expression of which blocks viral cell surface receptors, and should characterize the genetic factors responsible for resistance in *M. castaneus*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00301-14 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Mapping of Mouse Chromosomal Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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0.5

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We have been analyzing somatic cell hybrids and inter(sub)species crosses to develop a multilocus genetic map of the mouse largely comprised of expressed genes and pseudogenes. Most genes are mapped by the analysis of the progeny of two sets of genetic crosses, an interspecies backcross and an intersubspecies backcross. DNAs from these mice have been typed for over 750 loci about half of which have been previously positioned to permit mapping of newly defined genes to specific positions on the linkage map. These studies have resulted in the genetic mapping of several hundred new genes including, most recently, brain cDNAs, genes encoding bone morphogenetic proteins, opioid receptor proteins, tyrosine kinases, and sulfotransferases. Specific map locations can be useful information since proximity to a known developmental mutation can identify such a gene as a potential candidate for the abnormal phenotype. Thus, a gene for a new cartilage-derived morphogenetic factor was mapped to a site on Chromosome 2 near the mutation *bp* (brachypodism). Subsequent analysis demonstrated that the mutation was due to a specific abnormality of this locus. Other studies have focussed on the organization of multigene families in the mammalian genome and on the comparative linkage relationships of homologous genes in man and mouse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00304-14 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Retroviral Diseases

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3 ½

OTHER:

none

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigated a natural mechanism of resistance to retroviruses that occurs in mice and other species with a view toward whether a similar resistance could be devised for HIV in man. Mice that inherit a gene (designated *Fv4*) related to a retroviral envelope are resistant to infection with retroviruses that use a particular (ecotropic) virus receptor. It is believed that the resistance is due to blockage of the receptor by endogenously produced envelope protein. Such blockage could occur because of interaction of receptor with envelope inside the cell, as occurs in cells co-expressing the CD4 receptor for HIV and HIV envelope, or because of interaction of secreted envelope protein with the receptor on the surface of other cells. Consistent with the latter model, we found that a soluble form of Fv4 envelope (gp70) is secreted in serum and in supernatants of tissue culture cells expressing Fv4 envelope, and that this secreted envelope can block infection in vitro. This result may explain why mice transplanted with a mixture of Fv4-resistant and control bone marrow are partially resistant to infection with murine retroviruses. In a related tissue culture system for HIV, we found that when HIV envelope and vpu genes were co-expressed with the HIV receptor CD4, cell surface CD4 decreased due to rapid degradation of CD4 inside the cell. However, cells that initially expressed low levels of surface CD4 gave rise to cells with high surface CD4 and vice versa. A possible clue to the mechanism of inconstant down-regulation of CD4 is that drugs such as brefeldin A that block intracellular transport of proteins had markedly different effects on CD4 in cells depending on whether they also expressed HIV envelope and vpu. We are investigating models of the kinetics of synthesis, transport and vpu/env-mediated degradation of CD4 that may provide insights into the bimodal nature of surface CD4. We believe that better understanding of this phenomenon will suggest ways to enhance down-regulation of CD4 in order to enhance envelope-mediated resistance for HIV. We also worked on a new expression system for retroviral genes using Semliki Forest virus (SFV). We constructed SFV vectors encoding functional murine retroviral gag and env genes and are investigating possible uses of this expression system for retroviral vaccination and gene therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00415-11 LMM

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October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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2

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two potential animal models of HIV-1 induced disease have been evaluated. In the first, a chimpanzee was simultaneously inoculated with large amounts of three different primary HIV-1 isolates (HIV-1_{DH12}, HIV-1_{DH20}, and HIV-1_{DH29}) as both cell-free and cell-associated virus. During the primary infection, HIV-1_{DH12} was isolated directly from the plasma and HIV-1_{DH20} DNA was detected following amplification of PBMC DNA between weeks 9 and 11. Following resolution of the initial infection, the immune system of the infected chimpanzee was stimulated by two infusions of human PBMCs at weeks 23 and 28 post inoculation. This resulted in the appearance of HIV-1_{DH29} in lymphocytes recovered from a lymph node biopsy. The subsequent administration of high doses of steroids induced a plasma viremia at weeks 48 and 70, marked elevations of ELISA antibody levels, and increased numbers of PBMC carrying copies of HIV-1 DNA.

In the second, seven cynomolgus macaques, one rhesus macaque, and two pig-tailed macaques have been inoculated with the HIV-1/SIV chimeric virus (SHIV) that carries the *vpr-tat-rev-vpu-env-nef* gene region from the HIV-1_{DH12} isolate (and designated SHIVmad1). All of the monkeys became infected. One of cynomolgus monkeys exhibited significant drop of CD4+ cells (less than 100 cells/ μ l blood) which has persisted for more than a year. This monkey has recently developed a wasting syndrome beginning approximately 60 weeks after inoculation, which has resulted in a loss of more than 30% body weight. Some of the other monkeys have exhibited a transient drop of CD4 cells, and one of the pig-tailed macaques has experienced a gradual decrease of CD4 positive lymphocytes (16 weeks post inoculation). Two cynomolgus macaques have recently been transfused with blood from the animal with low CD4 cell levels. Since the SHIVs isolated from several of the infected monkeys replicate more rapidly and to higher titers than the original virus, several molecular clones corresponding to these viruses have been obtained and are being evaluated for infectivity in both tissue culture systems and *in vivo*. Some of the persistently infected macaques with relatively low virus load and no CD4 depletion are being treated with immunosuppressive agents in an attempt to increase levels of circulating SHIV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00527-08 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Cloning and Characterization of Human Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.0

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HIV-1 isolates can be divided into two major subgroups on the basis of their cellular host range in vitro: macrophage (MT) and T-cell line tropic. MT-tropic isolates infect both macrophages and peripheral blood mononuclear cells (PBMC) but are unable to replicate in transformed CD4+ T-cell lines. T-cell line tropic isolates infect both PBMC and CD4+ T-cell lines but replicate poorly or not at all in primary macrophages (MDM). Although T-cells are the major target for HIV-1 replication in peripheral blood, macrophages represent the predominant HIV-1 infected cell type in most tissues. Macrophages are probably the primary reservoir of HIV-1 and may be important for sustaining a persistent infection in individuals for many years. Most HIV-1 isolates we have cloned are T-cell tropic. We have succeeded in obtaining a complete molecular clone from a macrophage-tropic viral isolate. Preliminary biochemical and physical analyses have shown that the spontaneous shedding of the envelope protein(gp120) is drastically different from the typical T-cell line variants. Also the effects of several accessory genes (non-essential in in vitro infections) appear to be dispensable. Mutations in VPU, VPR, or NEF modestly reduced viral replication of the AD8-2 clone in either PBMC macrophages.

By comparison with other full-length infectious macrophage-tropic clones of HIV-1, the pAD8-directed HIV-1_{AD8} stock grew to high titers in cultures of human MDM, as monitored by p24 antigen capture assay. Progeny virion production in HIV_{AD8}-infected primary human macrophages was also readily measurable by the less sensitive reverse transcriptase (RT) assay.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00528-08 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Studies of HIV-1 Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL STAFF YEARS:

4

PROFESSIONAL:

4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the life cycle of HIV, the Rev protein regulates the temporal switch from the early regulatory to the late lytic phase by binding to a highly structured RRE (Rev Responsive Element) RNA. Our studies of Rev and RRE RNA were targeted: i) to analyze the various functional motifs of the Rev protein; and ii) to characterize the function of the putative cellular factors that may bind RRE RNA, Rev, or both. Since last year, we have further characterized the biochemical properties of the cellular ds RNA binding protein, TRBP that bound to the RRE# RNA. TRBP was a potent inhibitor of the interferon induced PKR kinase in vitro and in vivo. Both PKR and TRBP were capable of dimerizing and the PKR inhibition by TRBP was mediated by heterodimerization with PKR.

We have extended our studies on the effect of HIV-1 NEF protein on T lymphocyte CD4 receptor. We demonstrated that Nef downregulates CD4 expression through a bi-modal mechanism through endocytosis of cell surface receptor and repression of biosynthesis and transport of nascent CD4. Using the yeast two hybrid system of genetic screening, we identified two HeLa cell proteins that interact with Nef. One of these, RKA 29 is a polypeptide of 255 amino acids. The polypeptide has a basic N terminal end and a highly acidic C terminus that is critical for HIV Nef binding. RK29 is a nuclear protein and associates with the unmyristylated Nef protein in HeLa cells. The acidic C-terminal portion of RK29 has homologies with transcription factors suggesting that it is itself a transcription factor and may represent a cellular cofactor for Nef effects on HIV or non HIV transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00547-07 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanism(s) of Human Retrovirus trans-Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Molecular Microbiology

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8

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7

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1 (Trinh; student)

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Our research program continues to focus on three broad areas. These encompass: I) Molecular Regulation of HIV-1; II) Molecular Regulation of HTLV-I; and III) Molecular applications relevant for the development of HIV-1 specific ribozymes and transdominant/attenuated human immunodeficiency viruses. Here I summarize our progress over the past year. A more detailed visualization of our research is reflected in our annual bibliography. In brief, our research in 1994-1995 are described with the following 11 points.

Recent selected findings include: 1) progress in understanding the modulation of Sp1 phosphorylation by HIV-1 Tat; 2) the *in vivo* selection of Tat mutants through forced high passage of recombinant HIV-1 genomes containing insertion of a Tat cDNA into *nef*; 3) the demonstration of a physical interaction between HIV-1 Tat and cellular protein kinase, PKR; 4) the characterization of a cellular function for TAR-RNA-binding protein, TRBP; 5) the discovery of a physical interaction between secreted Tat protein and cellular growth factor, epithelin; 6) the development of chimeric HIV-1 and SIVmac nef- HSVtk+ viruses as possible viral vaccine strains; 7) the optimization of semliki forest virus as an anti-HIV-1 ribozyme vector; 8) the isolation and characterization of cDNAs that encode cellular proteins that bind to HTLV-I Tax using the yeast two hybrid system; 9) the definition of a novel repressive effect of HTLV-I Tax on the c-myc oncoprotein; 10) the subnuclear localization of the HTLV-I Tax protein using confocal microscopy; 11) the characterization of differences between HTLV-I and -II Tax proteins in the induction of micronuclei.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00669-03 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Retroviral Genes and their Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

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3.0

PROFESSIONAL:

3.0

OTHER:

0.0

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- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HIV is a complex retrovirus containing a number of genes not commonly found in other retroviruses. We are focusing on the analysis of two of these genes, vpu and vif. Vpu is encoded only by HIV-1 and encodes for a small integral membrane protein. Vpu is phosphorylated by casein kinase II at two highly conserved serine residues. Vpu regulates two biological functions: (i) enhancement of particle release, and (ii) degradation of CD4. To study the function of Vpu, we constructed a series of mutants and analyzed their effect both with regard to Vpu-mediated enhancement of particle release as well as CD4 degradation. We found that phosphorylation of Vpu was absolutely essential for CD4 degradation while unphosphorylated Vpu still retained at least 50% of wild type activity with regard to particle release. Phosphorylation of Vpu did not affect stability or intracellular distribution of Vpu. Thus, the two biological functions of Vpu are differentially regulated by phosphorylation. To study the importance of the Vpu TM domain for its function, we created a mutant containing a scrambled TM domain, VpuRD. In contrast to the Vpu phosphorylation mutant, VpuRD was still capable of inducing CD4 degradation although at a slightly reduced rate. However, this mutant was no longer able to support virus release from the cells. Preliminary evidence suggests that the Vpu TM domain forms an ion pore. It is thus possible, that virus release is regulated by an ion channel function of Vpu. In contrast, additional studies investigating the mechanism of CD4 degradation demonstrate that this process involves a direct interaction between Vpu and CD4. In summary, our data suggest that Vpu has two biological functions that are executed in different cellular compartments, rely on different functional domains of Vpu, and are mechanistically unrelated.

Vif is a 23 kDa basic protein which has an important function in regulating infectivity of progeny virions. The biochemical mechanism of Vif function is obscure. We analyzed the role of Vif by studying its subcellular distribution by cell fractionation as well as confocal microscopy. We found that approximately half of intracellular Vif protein is associated with the cytoskeleton, specifically intermediate filaments. The association of Vif with intermediate filaments is specific and can result in the reorganization of the cytoskeletal network. Experiments are ongoing to study the significance of this finding and to correlate it with the observed effects of Vif on viral infectivity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00715-02 LMM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

RNA-mediated Recombination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. K. Derr Senior Staff Fellow LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

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2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The reverse transcription of cellular RNAs and the subsequent insertion of the cDNA into the chromosome, a process referred to as RNA-mediated recombination, has probably played an important role in shaping the mammalian genome. The homologous or nonhomologous insertion of the reverse transcribed cellular transcript (cDNA) can provide a template for the evolution of new gene function, permit the development of alternative regulatory strategies and lead to chromosomal rearrangements. The purpose of this work is to understand the mechanism of RNA-mediated recombination and the viral and cellular functions required, using a yeast model system. We have shown that both the LTR-containing retrotransposon Ty and a LINE element can serve as sources of reverse transcriptase activity. These elements use very different priming mechanisms, and the mechanism of priming and insertion by LINE elements is not clearly understood. One hypothesis is that priming initiates from a nick in the chromosomal DNA. We have constructed a strain in which the topoisomerase I gene is overexpressed, to increase chromosomal nicking, as a tool for understanding priming and insertion of LINEs. A second aspect of the project is aimed at identifying the cellular genes involved in chromosomal insertion of the cDNA. We have developed an assay that specifically detects recombination between a diffusible cDNA and chromosomal allele. Using this assay, we have shown that RNA-mediated gene conversion is dependent on RAD52 (involved in most recombination) and RAD1 (involved in excision repair). Insertion of the cDNA can also be mediated by viral functions. We can detect reverse transcription and cDNA insertion of chromosomal transcripts using the retrotransposon Ty3. Ty3 inserts site-specifically upstream of tRNA genes. Therefore, Ty3 may provide a vehicle for gene delivery, eliminating concern for non-specific integration into the open reading frame of essential genes.

Laboratory of Molecular Structure
1995 Annual Report
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Annual Report
Laboratory of Molecular Structure
National Institute of Allergy and Infectious Diseases
October, 1, 1994 to September 30, 1995

RESEARCH PROGRESS

The Lab of Molecular Structure (LMS) investigates the structure and function of genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and the molecular mechanisms controlling T-cell development. Special emphasis is placed on membrane receptors and differentiation antigens, in particular the family of adhesion molecules known as integrins, present on T and B lymphocytes, on the structures of major histocompatibility genes and proteins, on the nature of the peptides bound by class I and class II molecules, on the molecular basis by which antigens are processed and presented to T cells, on the *cis* elements and *trans* factors that regulate T cell receptor and class I gene expression, the structural basis for the presentation to T cells of non peptide ligands by CD1 molecules, on natural killer (NK) cell receptors and their ligands, and on proteins of the complement system. The Structural Biology Section concentrates on determining the molecular structure and function of immunologically relevant molecules, such as cytokines and their receptors, and T cell receptors. In addition to this fundamental immunological research, the LMS provides protein sequencing, peptide synthesis, mass spectrometric, and flow cytometric expertise and services for the DIR.

STUDIES ON THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of intracellular proteins such as viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules and T cell receptors are emphasized in this research.

Peptide Binding Motifs Can Be Used to Predict Epitopes Involved in Disease. Identification of the targets of autoreactive T cells is important for understanding the pathogenesis of many autoimmune diseases. In multiple sclerosis, myelin proteins are thought to be the targets of autoreactive T-cell responses. To date, only major histocompatibility complex class II-restricted CD4⁺ T-cell responses to myelin proteins have been investigated. In the present study, the ability of self peptides derived from human myelin proteins to induce autoreactive CD8⁺ T-cell responses were assessed. Peptide sequences from human myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), and myelin oligodendrocyte glycoprotein have been identified that bind to and form stable complexes with HLA-A2. Several of these

peptides were able to induce peptide-specific HLA-A2-restricted CD8⁺ cytotoxic T-lymphocyte (CTL) responses *in vitro* in HLA-A2⁺ individuals. CTLs specific for MBP 110-118 and MAG 556-564 produced tumor necrosis factor α and a subset of these clones also produced interferon γ . These results demonstrate that (i) self peptides derived from human myelin proteins can induce autoreactive CD8⁺ CTLs and (ii) these CD8⁺ T cells produce cytokines thought to be important in mediating demyelinating disease. These studies provide an experimental approach for the assessment of CD8⁺ T-cell responses in such autoimmune diseases.

An ovalbumin (OVA)-specific T cell line (TCL) was established from a patient with hen egg allergy. This CD4⁺, $\alpha\beta$ TCR expressing TCL recognized OVA presented by HLA-DR10. Based on the response of the TCL to synthetic OVA peptides, it was found that the TCL recognized OVA 323-339. The TCL secreted high levels of IL-5, but undetectable amounts of IL-2, interferon- γ , and IL-4 when stimulated with OVA or the OVA 323-339 peptide. Since IL-5 is an important growth and chemotactic factor for eosinophils, it is possible that these OVA 323-339 specific T cells can contribute to human egg allergy. To our knowledge, this is the first demonstration of food allergen-specific TCL and identification of a T cell epitope possibly related to the allergic reaction to food antigens. An analog peptide of the OVA 323-339 partially inhibited the response of the TCL to OVA 323-339 presented by HLA-DR10, raising the possibility of peptide-based immunotherapy of food allergy.

Sequence Motifs Important for Binding to HLA-B14, HLA-B27 and DR15Dw2. Most peptides that bind to a particular major histocompatibility complex class I molecule share amino acid residues important for binding at one or two positions. Sequence analyses of peptides bound to HLA-B14 revealed at least four candidates for these so-called anchor residues: Arg at P2, Tyr at P3, Arg at P5, and Leu at P9. Combinations of any three of these amino acids sufficed for binding to HLA-B14 *in vitro*. Using this information, we identified an antigenic peptide critical for cytotoxic T lymphocyte recognition of virus-infected cells. Thus even when multiple combinations of anchor residues contribute to binding, antigenic peptides are routinely identifiable.

Antigenic peptides are presented to CD4⁺ T cells by MHC class II molecules via a highly polymorphic peptide-binding groove. The two HLA-DR alleles isotopically expressed on HLA-DR15Dw2-positive cells, DRB1*1501 (DR2b) and DRB5*0101 (DR2a) molecules, show a number of differences in polymorphic residues of the β -chain, including a Gly-Val-dimorphism at position β 86. Therefore, different requirements for interaction of peptides with the alleles are expected. In this study, naturally processed self-peptides were eluted from purified HLA-DR15Dw2 molecules and related to DRB1*1501 or DRB5*0101 molecules by binding assays. An alignment of self-peptides and foreign peptides allowed the delineation of putative anchor motifs. DRB5*0101 requires a bulky hydrophobic residue (F or Y) at position i as a primary anchor, and Q or an aliphatic residue, such as V, I, or M, at position i +3; positively charged residues at positions i + 7 and i + 8 are secondary anchors. For DRB1*1501, a nonaromatic, hydrophobic anchor (L, V, or I) at position i is supplemented by a bulky hydrophobic residue (F or Y) at position i + 3 as a primary anchor; an additional hydrophobic side chain represented by M, I, V, or F occurs at position i + 6. Because HLA-DR15 Dw2 is associated with susceptibility to develop multiple sclerosis, the delineation of ligand motifs of the two DR2 alleles may help to study the interaction between potential autoantigenic peptides and these molecules in the future. Analysis of a series of site-directed mutations in the heavy chain showed that drastic

structural changes in one subsite for peptide binding do not affect other subsites indicating that the dominant anchor residues at P2 and P9 of the peptide independently contribute to stabilizing the class I/peptide complex.

Adenovirus 12 (Ad12) Interference with Antigen Presentation Results in Tumors.

The expression of class I major histocompatibility complex antigens on the surface of cells transformed by adenovirus 12 (Ad12) is generally very low, and correlates with the high oncogenicity of this virus. In primary embryonal fibroblasts from transgenic mice that express both endogenous H-2 genes and a miniature swine class I gene (PD1), Ad12-mediated transformation results in suppression of cell surface expression of all class I antigens which is not due to decreased class I mRNA levels. Analysis of steady state mRNA levels of the TAP1 and TAP2 transporter genes for Ad12-transformed cell lines revealed that they both are significantly reduced. Reconstitution of PD1 and H-2D^b, but not H-2K^b, expression is achieved in an Ad12-transformed cell line by stable transfection with a TAP2, but not a TAP1, expression construct. From these data it may be concluded that suppressed expression of peptide transporter genes, especially TAP2, in Ad12-transformed cells inhibits cell surface expression of class I molecules. These results suggest that suppression of peptide transporter genes might be an important mechanism whereby virus-transformed cells escape immune recognition *in vivo*.

ROLE OF INTEGRINS IN THYMIC DEVELOPMENT

Merosin/Laminin Integrin Receptors May Play a Role in Thymocyte Development.

Integrins comprise a superfamily of $\alpha\beta$ heterodimers that serve as cell signaling as well as adhesion molecules. We demonstrated that $\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrins are laminin/merosin receptors expressed in human thymocytes. A small percentage (10 to 15%) of human thymocytes bind to immobilized laminin, and even fewer (3 to 5%) bind to merosin, the laminin isoform normally present in the thymus. This binding, however, can be increased to 39 to 41% after activation of thymocytes with Mn²⁺ (or PMA). The protein kinase C inhibitors, calphostin C and staurosporine, inhibit Mn²⁺-enhanced thymocyte binding, suggesting that protein kinase C activity is crucial for the binding. We also showed that both immobilized laminin and merosin have costimulatory function for anti-CD3-induced thymocyte proliferation, and both anti- α_3 and anti- α_6 mAbs can block this proliferative response. The cooperative function of $\alpha_3\beta_1$ and $\alpha_6\beta_1$ evidenced in the laminin/merosin binding and proliferation assays suggests that thymocyte-merosin interactions may play an important role in thymic T cell development.

Activation of β_1 Integrins Correlates with the Stage of T Cell Differentiation.

We demonstrated that the anti-mouse integrin β_1 chain mAb KMI6 selectively recognizes a β_1 epitope that is constitutively expressed by certain immature thymocytes and is induced only slightly on mature thymocytes and peripheral T cells by activation with Con A. Because virtually all cells examined expressed β_1 integrins on their surface, expression of the KMI6 epitope is T cell differentiation stage specific. Most CD3⁺CD4⁺CD8⁻ thymocytes were KMI6⁺, with the lowest level of staining observed on the earliest CD44⁺IL-2R⁻ cells within this subset. Expression was down-regulated during the CD3⁺CD4⁺CD8⁻ to CD3⁺CD4⁺CD8⁺ transition, and lost by the CD4⁺CD8⁺ stage. Mature single positive thymocytes and resting peripheral T cells were also KMI6⁻. In contrast with the loss of the epitope before TCR expression by other thymocytes, most CD3⁺CD4⁺CD8⁻ and certain CD8⁺ $\gamma\delta$ TCR⁺ thymocytes were KMI6⁺. The unique selectivity of KMI6 recognition of β_1 integrins,

and its selective enhancement of ligand binding suggest that β_1 integrin structure and factors that regulate β_1 integrin binding are correlated with the stage of T cell differentiation.

Vitronectin Receptor - Mediated Activation of T Cells The vitronectin receptor (VNR), an $\alpha_v\beta_3$ heterodimer, functions as a co-stimulatory molecule for the activation of a subset of $V\gamma 1.1/C\gamma 4$ -bearing $\gamma\delta$ T cells, which has been postulated to recognize a ubiquitous self-antigen. We addressed the question of whether stimulation of these T cells requires both engagement of the VNR by ECM proteins and engagement of the TCR by its Ag. We introduced a TCR⁻ but VNR⁺ mutant T cell hybridoma, a chimeric molecule that contains the cytoplasmic tail of the TCR ζ -chain fused to the cytoplasmic and transmembrane region of either human CD8 or human CD25. The transfectants expressing the chimeric molecules secreted IL-2 constitutively when the VNR was engaged with its ligand. This constitutive cytokine secretion could be blocked with mAb directed against the VNR, or with the peptide RGD, or by growth in serum-free medium. Signaling through the ζ -chain was required, as cells transfected with a chimera containing only a 22 amino-acid long, truncated ζ -chain did not secrete IL-2 constitutively. Thus, we demonstrated that the binding of the VNR to ECM protein in the presence of the ζ -chain is sufficient to induce cytokine secretion by T cells and does not require recognition of an Ag by the TCR. Such integrin-mediated, Ag-independent activation of T cells may play a critical role in the potentiation of inflammatory responses.

X-RAY CRYSTALLOGRAPHY

Crystal Structure Determination of Proteins

We are currently focusing on the crystallographic characterizations of the following proteins; 1) a soluble human type II TGF- β receptor; 2) soluble Fc γ receptors; 3) a molybdopterine containing formate dehydrogenase H (FDH) and 4) blood type converting enzymes. We have crystallized the human soluble type II TGF- β receptor in complex with TGF- β 1, achieved preliminary expression of the soluble Fc γ receptors, CD16 and CD32, in a CHO cell expression system. We have also obtained crystals of TGF- β 1 in complex with a TGF- β 1 neutralizing antibody 1D11.16. The crystallization of E.coli FDH and a A- to O- blood type converting enzyme, α -N-acetylgalactosaminidase (α -NAGal), both yielded diffraction quality crystals. To determine the crystal structure of FDH, we have collected a set of native data, several sets of heavy atom derivative data with known heavy atom binding sites and a multiple anomalous dispersion data.

In the case of α -NAGal, we have collected several native data sets and a number of heavy atom soaking data sets in the process of searching suitable derivatives for MIR (multiple isomorphous replacement) phasing.

PROTEINS OF THE COMPLEMENT SYSTEM

Sgp120 was first identified by its capacity to interact with the activated fourth component (C4b) of the classical complement pathway (CCP). The full-length cDNA for sialoglycoprotein (sgp120) was elucidated from human fetal liver cDNA libraries by standard cloning and sequencing technologies and it was demonstrated that several forms of the mRNA that codes for sgp120 exist due to spliced variants and Sgp120 is also cleaved by kallikrein of the intrinsic coagulation-kinin generating pathway to produce protein fragments that may

possess biological activity and the data supports our hypothesis that the larger C4b binding form, sgp120-A, is a product of spliced mRNA. Using a sensitive ELISA for sgp120 we have identified 2-3 fold increases in the serum sgp120 levels of burn patients suggesting that sgp120 like other complement components is an acute phase reactant.

SYNTHESIS OF PEPTIDES

During the past year, almost 600 peptides have been produced by the Laboratory. Many have been used for defining MHC class I restricted T cell epitopes e.g. for influenza and myelin basic protein (multiple sclerosis), and for defining the nature of the interaction between class I molecules and peptides, to study mechanisms of antigen processing, to examine the specificity of natural killer (NK) cell receptors, and to map antibody epitopes. Peptides have also been used for preparing antisera to a large variety of proteins including integrins, NK receptors (NKG2), Fc receptors, virus proteins, human histamine I receptor, gag polypeptide of a neurovirulent murine retrovirus, human ZAP-70, purinergic receptor, RAG-2, thymidine kinase, OX-40, feline calicivirus protease, α -tubulin I, HIV-Rex, noval viral gene (VZV), and various oncogenes such as JUN and REL. Peptides have also been used to study the functional activity of HIV Tax and Rev proteins, malaria parasite invasion, Ras-related proteins in the activation of NADPH oxidase, B cell transcription factors, NK recognition, NF- κ B and I κ B, trafficking of malaria proteins, pathogenic scrapie-associated PrP, MuLV infection, vaccinia proteins, Kaposi sarcoma virus proteins, MAIDS viral proteins, HIV replication, and hepatitis E proteins. Peptides have also been used to study disease related T cell epitopes for rheumatoid arthritis, multiple sclerosis, experimental allergic encephalomyelitis (EAE), experimental myasthenia gravis, autoimmune gastritis and Schistosoma mansoni.

PROTEIN SEQUENCE ANALYSES

The LMS determined the amino acid sequences of about 550 samples. About 270 of these analyses were for unknown proteins or peptides and the remainder were for quality control of synthetic peptides. Most of the unknown sequences determined were of endogenous peptides eluted from human MHC class I (LMS) and class II molecules. From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information has been used to identify potential antigenic T cell epitopes that are recognized by cytotoxic and helper T lymphocytes. Knowledge of such epitopes is important for discerning disease processes and of potential importance for vaccine development. Using such an approach several new antigenic epitopes recognized by T cells specific for influenza virus proteins and myelin basic proteins have been identified. In addition, proteins and peptides from a large variety of other sources have been sequenced by the facility: These include eosinophil derived proteins (H. Rosenberg, LHD); neutrophil p7 phox (T. Leto, LHD); Duffy antigen (C. Chitnis, LPD); malaria proteins (D. Kaslow, LPD); membrane antigen of basophilic cells (J.P. Kinet, MAIS); and Streptococcal Group B hyaluronate lyase, hippuricase and endopeptidase (D. Pritchard, Univ. of Alabama at Birmingham).

Group B streptococci (GBS) are a major cause of serious human perinatal infections. Most clinical isolates of GBS secrete hyaluronate lyase, and production of high levels of the enzyme has been associated with strain virulence. Degenerate oligonucleotide primers, designed on the basis of the amino acid sequences of tryptic peptides prepared from the purified enzyme led to cloning of the gene from a λ phage library of GBS chromosomal DNA fragments. When this gene was transformed into *Escherichia coli*, high level expression of hyaluronate lyase activity was obtained.

A method was described by which a sequence-dependent peptide fingerprint can be rapidly obtained upon partial hydrolysis of peptide with hydrochloric acid and subsequent analysis by matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). When synthetic peptides are treated with 3M HCl for 5 min at 110°C, amino acids are released in turn from the C-terminus or, depending on the peptide, from the N-terminus. Sequence information can be deduced by identifying the amino acid residue whose mass corresponds to the difference in MW between the major hydrolysis products, beginning from the MW of the starting peptide. The technique we have developed can be used to obtain a sequence-dependent "fingerprint" for a peptide cheaply and rapidly, starting with the picomole amounts of peptide, because the hydrolysate can be directly analyzed by MALDI-MS.

FLOW CYTOMETRIC ANALYSES

The BD multiparameter FACS Vantage was installed this FY. It is equipped with three lasers (argon, argon/dye and krypton) which will enable it to perform 5 visible colors, and combined visible/UV analyses and sorting. It also has the four color-single laser option for performing 4 color analyses from the argon laser. Final stages of testing are being conducted to enable the FACStar Plus (and eventually the FACS Vantage) to perform analysis and sorting based upon 5 colors. This requires the use of interlaser compensation, previously unavailable. The amount of usage by DIR labs has increased from 3658.9 total hours in FY '94 to 4236 hours in FY '95. The percentage time devoted to sorting is expected to increase with the availability of the FACS Vantage.

Administrative Report

In March of this year, the *Laboratory of Molecular Structure* relocated from the Bethesda NIH campus to Twinbrook II in Rockville.

The *Molecular Immunology Section* saw the addition of Dr. Phillip Posch from Georgetown University, Dr. Edgar Fernandez from the Centro de Biologia Molecular "Severo Ochoa" of Madrid, Spain, Dr. Francesca Zappacosta from the University of Naples, Italy, Dr. Jorge Ochoa-Garay from the University of Southern California, Dr. Francisco Borrego from the University of Cordoba, Cordoba, Spain, Dr. JaeHun Cheong from Pusan National University, Korea, and Dr. Xiaohong Hou of the University of Copenhagen, Denmark. Dr. Rachel Ehrlich from Tel Aviv University arrived to do a six month sabbatical. Dr. Frederic Martinon returned to the Institut Cochin de Genetique Moleculaire, Paris, France. Dr. Knut Sturmhoefel assumed a position with Genetics Institute in Boston, Massachusetts, Dr. Yumiko Shirakata returned to Japan to assume a position with the National Institutes of Health, Japan, and Dr. Andrew Chang accepted a tenure-track position in the FDA.

The *Structural Biology Section* saw the departure of Dr. Maria Cueto who assumed a position with the Sandoz Pharmaceutical Corporation.

No changes occurred in the *Biological Resources Section*.

Honors and Awards

Dr. Coligan was invited to present research seminars at the Center for Disease Control in Atlanta, the University of Alabama at Birmingham, Johns Hopkins University, and the Roswell Park Memorial Cancer Institute. Dr. Coligan also presented the First Annual Fong/Clontech Lecture at Indiana University. Dr. Coligan made invited presentations at the Fourth International Workshop on MHC Evolution, American Association of Immunologists Annual Meeting, and the International Congress of Immunology; and served on the Expert Panel on Immunologic Intervention for Promoting Transplant Tolerance-State of the Art and Future Directions. Dr. Coligan continues to serve on the editorial boards of Current Protocols in Immunology, Molecular Immunology, Immunologic Research, Current Protocols in Protein Science and the Journal of Biomedical Science. He was appointed as a Cutting Edge Editor for the Journal of Immunology. Dr. Coligan serves on the Board of the Structural Biology Interest Group at the NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00169-18 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan, Ph.D., Chief, LMS, DIR, NIAID

Others:

A. Brooks V. Fellow, LMS, NIAID

J. Shuman Sr. Staff Fellow, LMS, NIAID

H. Chen IRTA LMS, NIAID

Y. Shirakata V. Associate, LMS, NIAID

J. Cheong V. Fellow LMS, NIAID

F. Zappacosta V. Fellow, LMS, NIAID

J. Ochoa-Garay V. Fellow, LMS, NIAID

K. Parker Sr. Staff Fellow, LMS, NIAID

COOPERATING UNITS (if any)

NeuroImmunology Branch, NINDS (W. Biddison); Univ. of Minnesota (J.P. Houchins); Univ. of Tubingen (R. Martin)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook II, Rockville MD. 20852

TOTAL STAFF YEARS:

4.4

PROFESSIONAL:

3.9

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Major histocompatibility complex (MHC) class I molecules bind endogenously synthesized peptides for presentation to cytotoxic T cells. The human class I molecule HLA-B27 consists of a trimolecular complex containing the HLA-B27 heavy chain, a peptide that is usually nine amino acid residues (aa) long, and β_2 -microglobulin (β_2m). The key interactions for peptide selectivity are between Glu-45, which forms a salt bridge with the Arg at P2 of the peptide and Asp-116 which favors the binding of peptides containing a Lys or Arg at P9. The $t_{1/2}$ of dissociation of $[^{125}I]\beta_2m$ was measured for peptide-specific HLA-B27 wild-type (wt) and mutant complexes. HLA-B27 wt and HLA-B27D116F formed relatively stable complexes, with a $t_{1/2}$ of dissociation on the scale of hours, with appropriate peptides that contained Arg at P2, whereas HLA-B27 E45T required a Gln at P2. Similarly, kinetically stable D116F complexes were formed only with peptides that contained a Leu or Val at P9 instead of Arg or Lys. The $[^{125}I]\beta_2m$ dissociation rate data were fit to a set of equations in order to calculate relative binding coefficients for each anchor residue at P2 and P9. The P2 coefficients were sensitive only to the D116F mutation. Thus, drastic structural changes in one subsite do not affect the other subsite, indicating that the dominant anchor residues at P2 and P9 independently contribute to stabilizing the class I/peptide complex.

Antigenic peptides are presented to CD4⁺ T cells by MHC class II molecules via a highly polymorphic peptide-binding groove. The two HLA-DR alleles isotopically expressed on HLA-DR15Dw2-positive cells, DRB1*1501 (DR2b) and DRB5*0101 (DR2a) molecules, show a number of differences in polymorphic residues of the β -chain, including a Gly-Val-dimorphism at position β 86. Therefore, different requirements for interaction of peptides with the alleles are expected. In this study, naturally processed self-peptides were eluted from purified HLA-DR15Dw2 molecules and related to DRB1*1501 or DRB5*0101 molecules by binding assays. An alignment of self-peptides and foreign peptides allowed the delineation of putative anchor motifs. DRB5*0101 requires a bulky hydrophobic residue (F or Y) at position i as a primary anchor, and Q or an aliphatic residue, such as V, I, or M, at position i +3; positively charged residues at positions i + 7 and i + 8 are secondary anchors. For DRB1*1501, a nonaromatic, hydrophobic anchor (L, V, or I) at position i is supplemented by a bulky hydrophobic residue (F or Y) at position i + 3 as a primary anchor; an additional hydrophobic side chain represented by M, I, V, or F occurs at position i + 6. Because HLA-DR15 Dw2 is associated with susceptibility to develop multiple sclerosis, the delineation of ligand motifs of the two DR2 alleles may help to study the interaction between potential autoantigenic peptides and these molecules in the future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00172-17 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Peptide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan, Ph.D., Chief, LMS, DIR, NIAID

Others:

Carl Hammer, Ph.D., Senior Investigator, LMS, DIR, NIAID

Jan Lukszo, Ph.D., Senior Staff Fellow, LMS, NIAID

COOPERATING UNITS (if any)

Centers for Disease Control (Renu Lal); NICHD (K. Ozato); USUHS (S. Vogel); Tel Aviv University, Israel (Rachel Ehrlich)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Biological Resources Section

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook II, Rockville, MD 20852

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

2.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year, almost 600 peptides have been produced by the laboratory. Many have been used for defining MHC class I restricted T cell epitopes e.g. for influenza and myelin basic protein (multiple sclerosis), and for defining the nature of the interaction between class I molecules and peptides, to study mechanisms of antigen processing, to examine the specificity of natural killer (NK) cell receptors, and to map antibody epitopes. Peptides have also been used for preparing antisera to a large variety of proteins including integrins, NK receptors (NKG2), Fc receptors, virus proteins, human histamine I receptor, gag polypeptide of a neurovirulent murine retrovirus, human ZAP-70, purinergic receptor, RAG-2, thymidine kinase, OX-40, feline calicivirus protease, α -tubulin I, HIV-Rex, noval viral gene (VZV), and various oncogenes such as JUN and REL. Peptides have also been used to study the functional activity of HIV Tax and Rev proteins, malaria parasite invasion, Ras-related proteins in the activation of NADPH oxidase, B cell transcription factors, NK recognition, NF- κ B and I κ B, trafficking of malaria proteins, pathogenic scrapie-associated PrP, MuLV infection, vaccinia proteins, Kaposi sarcoma virus proteins, MAIDS viral proteins, HIV replication, and hepatitis E proteins. Peptides have also been used to study disease related T cell epitopes for rheumatoid arthritis, multiple sclerosis, experimental allergic encephalomyelitis (EAE), experimental myasthenia gravis, autoimmune gastritis and Schistosoma mansoni.

The expression of class I major histocompatibility complex antigens on the surface of cells transformed by adenovirus 12 (Ad12) is generally very low, and correlates with the high oncogenicity of this virus. In primary embryonal fibroblasts from transgenic mice that express both endogenous H-2 genes and a miniature swine class I gene (PD1), Ad12-mediated transformation results in suppression of cell surface expression of all class I antigens. Although class I mRNA levels of PD1 and H-2D^b are similar to those in nonvirally transformed cells, recognition of newly synthesized class I molecules by a panel of monoclonal antibodies is impaired, presumably as a result of inefficient assembly and transport of the class I molecules. Analysis of steady state mRNA levels of the TAP1 and TAP2 transporter genes for Ad12-transformed cell lines revealed that they both are significantly reduced. Reconstitution of PD1 and H-2D^b, but not H-2K^b, expression is achieved in an Ad12-transformed cell line by stable transfection with a TAP2, but not a TAP1, expression construct. From these data it may be concluded that suppressed expression of peptide transporter genes, especially TAP2, in Ad12-transformed cells inhibits cell surface expression of class I molecules. These results suggest that suppression of peptide transporter genes might be an important mechanism whereby virus-transformed cells escape immune recognition *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00352-13 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan, Ph.D., Chief, LMS, DIR, NIAID

Others:

A. Chang, Ph.D., IRTA, LMS, NIAID

E. Fernandez, Ph.D., Visiting Fellow, LMS, NIAID

M. Halvorsen, Ph.D., IRTA, LMS, NIAID

W. Magner, Ph.D., IRTA, LMS, NIAID

F. Martinon, Ph.D., IRTA, LMS, NIAID

S. Otto, Ph.D., IRTA, LMS, NIAID

COOPERATING UNITS (If any)

Laboratory of Immunology, NIAID (Ethan Shevach); RW Johnson Pharmaceuticals Research Institute (Scott Wadsworth)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Biological Resources Section

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook II, Rockville, MD 20852

TOTAL STAFF YEARS:

5.4

PROFESSIONAL:

4.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We demonstrated that $\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrins are laminin/merosin receptors expressed in human thymocytes. A small percentage (15%) of human thymocytes bind to immobilized laminin, and even fewer bind to merosin, the laminin isoform normally present in the thymus. This binding, however, can be increased to 39 to 41% after activation of thymocytes with Mn^{2+} (or PMA). Both $\alpha_3\beta_1$ and $\alpha_6\beta_1$ were shown to participate in thymocyte binding to laminin/merosin. Protein kinase C inhibitors inhibited Mn^{2+} -enhanced thymocyte binding, suggesting that protein kinase C activity is crucial for the binding. We also showed that both immobilized laminin and merosin have costimulatory function for anti-CD3-induced thymocyte proliferation, and both anti- α_3 and anti- α_6 mAbs can block this proliferative response. The cooperative function of $\alpha_3\beta_1$ and $\alpha_6\beta_1$ evidenced in the laminin/merosin binding and proliferation assays suggests that thymocyte-merosin interactions may play an important role in thymic T cell development.

We demonstrated that the anti-mouse integrin β_1 chain mAb KMI6 selectively recognizes a β_1 epitope that is constitutively expressed by certain immature thymocytes and is induced only slightly on mature thymocytes and peripheral T cells by activation with Con A. Because virtually all cells examined expressed β_1 integrins on their surface, expression of the KMI6 epitope is T cell differentiation stage specific. Most CD3⁺4⁺8⁺ thymocytes were KMI6⁺, with the lowest level of staining observed on the earliest CD44⁺ IL-2R⁻ cells within this subset. Expression was down-regulated during the CD3⁺4⁺8⁺ to CD3⁺4⁺8⁺ transition, and lost by the CD4⁺8⁺ stage. Mature single positive thymocytes and resting peripheral T cells were also KMI6⁺. The unique selectivity of KMI6 recognition of β_1 integrins, and its selective enhancement of ligand binding suggest that β_1 integrin structure and factors that regulate β_1 integrin binding are correlated with the stage of T cell differentiation.

The vitronectin receptor (VNR) functions as a co-stimulatory molecule for the activation of a subset of V γ 1.1/C γ 4-bearing $\gamma\delta$ T cells. We addressed the question of whether stimulation of these T cells requires both engagement of the VNR by ECM proteins and engagement of the TCR by its Ag. We introduced a TCR⁻ but VNR⁺ mutant T cell hybridoma, TG40, a chimeric molecule that contains the cytoplasmic tail of the TCR ζ -chain fused to the cytoplasmic and transmembrane region of either human CD8 or human CD25. The transfectants expressing the chimeric molecules secreted IL-2 constitutively when the VNR was engaged with ECM proteins. This demonstrates that the binding of the VNR to ECM protein in the presence of the ζ -chain is sufficient to induce cytokine secretion by T cells and does not require recognition of an Ag by the TCR. Such integrin-mediated, Ag-independent activation of T cells may play a critical role in the potentiation of inflammatory responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00522-08 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Sequence Analyses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan, Ph.D., Chief, LMS, DIR, NIAID

COOPERATING UNITS (if any)

German Cancer Research Ctr. (G. Hammerling), LHD/NIAID (H. Rosenberg and T. Leto), University of Leiden (F. Ossendorp and F. Koning), Univ. of Alabama (D. Pritchard), LPD/NIAID (C. Chitnis and D. Kaslow), MAIS/NIAID (J.P. Kinet)

LAB/BRANCH

Lab of Molecular Structure

SECTION

Biological Resources Section

INSTITUTE AND LOCATION

NIAID, NIH, Rockville, MD 20852

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The LMS determined the amino acid sequences of about 550 samples. About 270 of these analyses were for unknown proteins or peptides and the remainder were for quality control of synthetic peptides. Most of the unknown sequences determined were of endogenous peptides eluted from human MHC class I (LMS) and class II molecules. From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information has been used to identify potential antigenic T cell epitopes that are recognized by cytotoxic and helper T lymphocytes. Knowledge of such epitopes is important for discerning diseases processes and of potential importance for vaccine development. Using such an approach several new antigenic epitopes recognized by T cells specific for influenza virus proteins and myelin basic proteins have been identified. In addition, proteins and peptides from a large variety of other sources have been sequenced by the facility: These include eosinophil derived proteins (H. Rosenberg, LHD); neutrophil p7 phox (T. Leto, LHD); Duffy antigen (C. Chitnis, LPD); malaria proteins (D. Kaslow, LPD); membrane antigen of basophilic cells (J.P. Kinet, MAIS); and Streptococcal Group B hyaluronate lyase, hippuricase and endopeptidase (D. Pritchard, Univ. of Alabama at Birmingham).

Group B streptococci (GBS) are a major cause of serious human perinatal infections. Most clinical isolates of GBS secrete hyaluronate lyase, and production of high levels of the enzyme has been associated with strain virulence. Degenerate oligonucleotide primers, designed on the basis of the amino acid sequences of tryptic peptides prepared from the purified enzyme led to cloning of the gene from a λ phage library of GBS chromosomal DNA fragments. When this gene was transformed into *Escherichia coli*, high level expression of hyaluronate lyase activity was obtained.

A method was described by which a sequence-dependent peptide fingerprint can be rapidly obtained upon partial hydrolysis of peptide with hydrochloric acid and subsequent analysis by matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). When synthetic peptides are treated with 3M HCl for 5 min at 110°C, amino acids are released in turn from the C-terminus or, depending on the peptide, from the N-terminus. Sequence information can be deduced by identifying the amino acid residue whose mass corresponds to the difference in MW between the major hydrolysis products, beginning from the MW of the starting peptide. The technique we have developed can be used to obtain a sequence-dependent "fingerprint" for a peptide cheaply and rapidly, starting with the picomole amounts of peptide, because the hydrolysate can be directly analyzed by MALDI-MS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00523-08 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Flow Cytometric Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kevin L. Holmes Head, Flow Cytometry Unit

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Flow Cytometry Unit

INSTITUTE AND LOCATION

NIAID

TOTAL STAFF YEARS:

5.25

PROFESSIONAL:

0.25

OTHER:

5.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Flow cytometry facility has recently installed a FACSCan for use by DIR investigators; this instrument is being rented from the BEIB at NIH. In addition to its use by the DIR, we will use it to provide a theory and practical course in flow cytometry.

The BD multiparameter FACS Vantage was installed this FY. It is equipped with three lasers (argon, argon/dye and krypton) which will enable it to perform 5 visible colors, and combined visible/UV analyses and sorting. It also has the four color-single laser option for performing 4 color analyses from the argon laser.

Final stages of testing are being conducted to enable the FACStar Plus (and eventually the FACS Vantage) to perform analysis and sorting based upon 5 colors. This requires the use of interlaser compensation, previously unavailable.

The amount of usage by DIR labs has increased from 3658.9 total hours in FY '94 to 4236 hours in FY '95. The percentage time devoted to sorting is expected to increase with the availability of the FACS Vantage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00543-08 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recognition of Peptide Antigens by Virus-Specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan, Ph.D., Chief, LMS, DIR, NIAID

Others:

F. Borrego, Visiting Fellow, LMS/NIAID

M. DiBrino, IRTA LMS/NIAID

K. Parker, Sr. Staff Fellow, LMS/NIAID

P. Posch, IRTA LMS/NIAID

M. Shields, IRTA LMS/NIAID

COOPERATING UNITS (if any)

Neuroimmunology Branch, NINCDS (W. Biddison); Laboratory of Immunology, NIAID (D. Margulies), Chiba University, Japan (N. Shimojo)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook II, Rockville, MD 20852

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Identification of the targets of autoreactive T cells is important for understanding the pathogenesis of many autoimmune diseases. In multiple sclerosis, myelin proteins are thought to be the targets of autoreactive T-cell responses. In the present study, the ability of self peptides derived from human myelin proteins to induce autoreactive CD8⁺ T-cell responses were assessed. Peptide sequences from human myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), and myelin oligodendrocyte glycoprotein have been identified that bind to and form stable complexes with HLA-A2. MBP 110-118, PLP 80-88, MAG 287-295, MAG 509-517, and MAG 556-564 were all able to induce peptide-specific HLA-A2-restricted CD8⁺ cytotoxic T-lymphocyte (CTL) responses *in vitro* in HLA-A2⁺ individuals. CTLs specific for MBP 110-118 and MAG 556-564 produced tumor necrosis factor α and a subset of these clones also produced interferon γ . These results demonstrate that (i) self peptides derived from human myelin proteins can induce autoreactive CD8⁺ CTLs and (ii) these CD8⁺ T cells produce cytokines thought to be important in mediating demyelinating disease. These studies provide an experimental approach for the assessment of CD8⁺ T-cell responses in such autoimmune diseases.

Most peptides that bind to a particular major histocompatibility complex class I molecule share amino acid residues important for binding at one or two positions. Sequence analyses of peptides bound to HLA-B14 revealed at least four candidates for these so-called anchor residues: Arg at P2, Tyr at P3, Arg at P5, and Leu at P9. Combinations of any three of these amino acids sufficed for binding to HLA-B14 *in vitro*. Using this information, we identified an antigenic peptide critical for cytotoxic T lymphocyte recognition of influenza virus-infected cells. Molecular models of HLA-B14 peptide complexes were constructed to investigate how the potential anchor residues might function.

An ovalbumin (OVA)-specific T cell line (TCL) was established from a patient with hen egg allergy. The TCL was CD4⁺, expressed an $\alpha\beta$ T cell receptor, and recognized OVA presented by HLA-DR10. Based on the response of the TCL to synthetic OVA peptides, it was found that the TCL recognized OVA 323-339. The TCL secreted high levels of IL-5, but undetectable amounts of IL-2, interferon- γ , and IL-4 when stimulated with OVA or the OVA 323-339 peptide. Since IL-5 is an important growth and chemotactic factor for eosinophils, it is possible that these OVA 323-339 specific T cells can contribute to human egg allergy. To our knowledge, this is the first demonstration of food allergen-specific TCL and identification of a T cell epitope possibly related to the allergic reaction to food antigens. An analog peptide of the OVA 323-339, which is known to strongly bind to I-A^d, partially inhibited the response of the TCL to OVA 323-339 presented by HLA-DR10, raising the possibility of peptide-based immunotherapy of food allergy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00595-05 LMS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification, Characterization and Function of Proteins of the Complement System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Hammer, Senior Investigator, LMS, NIAID

Others:

M. Knierman, Chemist, PRI

Thomas Leto, Sr. Staff Fellow, LHD, NIAID

COOPERATING UNITS (if any)

Michael M. Frank, Chairman, Dept. Pediatrics, Duke Univ. Med. Ctr. Durham, NC 27710

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Biological Resource Section

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook II, Rockville, MD 20852

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The full-length cDNA for a new plasma sialoglycoprotein (sgp120) was elucidated from human fetal liver cDNA libraries by standard cloning and sequencing technologies. We have demonstrated that several forms of the gene that codes for sgp120 exist due to spliced variants. Sgp120 was first identified by its capacity to interact with the activated fourth component (C4b) of the classical complement pathway (CCP). Results support the idea that this form of the protein, sgp120-A, regulates the activation of the CCP. Sgp120 is also cleaved by kallikrein of the intrinsic coagulation-kinin generating pathway to produce protein fragments that may possess biological activity. The data supports our hypothesis that the larger C4b binding form, sgp120-A, is a product of a spliced gene. Pigtryin (PT), an identified acute phase protein and postulated analog of human inter-alpha-trypsin inhibitor (IATI) was shown to have a peptide match of 76% identity with 81% similarity with sgp120. Thus, PT is most likely the pig analog of human sgp120 and not IATI. Using a sensitive ELISA for sgp120 we have identified 2-3 fold increases in the serum sgp120 levels of burn patients suggesting that sgp120 like other complement components is an acute phase reactant. Sgp120 also shows strong homology to each of the three mature heavy chains of IATI. Homology to HC3 was most significant with 51% identity and 71% similarity over the 5' 619 amino acid residues upstream from the splice region.

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter Sun, Ph.D., Sr. Staff Fellow, LMS, NIAID

Others:

L. Hannick, Ph.D., Sr. Staff Fellow, LMS, NIAID

J. Boyington, Ph.D., IRTA Fellow, LMS, NIAID

Y. Zhang, Sr. Staff IRTA Fellow, LMS, NIAID

COOPERATING UNITS (if any)

Sandoz (M. Cueto); R&D Systems (M. Tsang0; Lab of Chemoprevention, NCI, (S-J Kim); Celtrix (Y. Ogawa); Lab of Biochem, NHLBI (T. Stadtman); Mount Sinai School of Med. (J. Unkeless); New York Blood Center (A. Zhu)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Structural Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook II, Rockville, MD 20852

TOTAL STAFF YEARS:

3.8

PROFESSIONAL:

3.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. TGF- β related proteins. We have crystallized a TGF- β I complex with a neutralizing antibody ID11.16 in order to delineate the surface loops of TGF- β I that mediate the receptor binding. The best crystals are grown in the presence of 8 - 10% PEG 8000, 20% glycerol, 0.2 M sodium acetate and 0.1 M sodium citrate at pH 5.6. A typical crystal measures 0.1 x 0.1 x 0.3 mm in size and it diffracts to about 2.9 Å resolution. Using SDS gel electrophoresis, we have shown the presence of both TGF- β I and ID11.16 in this crystal form. We have also crystallized a complex of TGF- β and its type II receptor. The existence of the complex has been demonstrated by SDS gel electrophoresis. Currently, the size of these crystals are still small and further improvement in crystallization condition is needed to obtain data collection quality crystals.

B. Immunoglobulin Fc receptors. A method has been developed in this laboratory to over express soluble Fc receptors of human Fc γ RII, Fc γ RIII and a mouse Fc γ RII. Cells are cultured on the surface of cytodex 2 beads as microcarriers. A three step scale-up protocol is implemented in order to obtain milligram quantity of proteins.

C. Molybdopterin containing formate dehydrogenase H from E. coli. Due to the extreme oxygen sensitivity of FDH, the enzyme was purified and crystallized under anaerobic conditions. Crystals suitable for X-ray diffraction studies were obtained in a space group P4₂/2 with cell dimensions a=b=146.2 Å, c=83.2 Å. We have developed a cryofreezing protocol for the FDH data collection to avoid oxidation of the enzyme. The native and a number of heavy atom derivative screening data sets have been collected using our lab source x-ray facilities. Among the potential heavy atom derivatives, we solved a major binding site of K₂PtCl₄ by inspecting its difference Patterson map and other derivatives by cross Fourier method. A set of multiple wavelength diffraction data were collected using Brookhaven synchrotron facility for phase determinations.

D. Blood-type converting enzymes. We have crystallized two blood-type converting enzymes, an α -galactosidase (α -Gal) which converts A- to O-type blood and an α -N-acetylgalactosaminidase (α -NAGal) that converts B- to O-type blood. Crystals of α -NAGal grow to a size of 0.85mm x 0.28mm x 0.22mm from a solution containing 1.5M ammonium sulfate or lithium sulfate and 0.1 M sodium acetate at pH 5.6. They diffract to 2.8 Å and belong to the space group P4₂/2 with unit cell dimensions of a=b=72.1 Å, c=176.3 Å (α = β = γ =90°). Native and a number of heavy atom derivative data sets have been collected under cryofreezing conditions both in our lab and in NSLS for MIR and anomalous phase determination.

LABORATORY OF PARASITIC DISEASES
1995 Annual Report
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LABORATORY OF PARASITIC DISEASES

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

This has been a significant year for the parasitology laboratories at the NIH, primarily because of the reunion of the Laboratory of Parasitic Diseases and the Laboratory of Malaria Research. Historically, parasitology research within the NIAID has undergone repeated divisions and fusions. Originally under Willard Wright, all programs were included in one laboratory. However, in 1959, the Laboratory was divided to provide leadership opportunities for two strong individuals, Leon Jacobs and G. Robert Coatney. One of John Seal's early and wise decisions as NIAID Scientific Director was to reunite the two in 1969 into a single Laboratory of Parasitic Diseases under Franklin Neva.

In 1992, an independent Laboratory of Malaria Research was formed. The LPD and the newly formed LMR continued to work closely, sharing a joint seminar series and common equipment, and when Franklin Neva decided to step down as the Chief of the Laboratory of Parasitic Diseases to focus on his own research (after a short detour as Acting Scientific Director), the decision was made by the NIAID Director to bring the two Laboratories back together. In January 1995, this reunion was made official as a single Laboratory of Parasitic Diseases; Louis Miller was named Chief of the new laboratory.

In the last few years, five tenured scientists have left the Laboratory of Parasitic Diseases. Two of these, Eric Ottesen and Allen Cheever, both Section Heads departed during this reporting period. A major effort of the last year has been the search for a scientist to lead a new program in protozoan biochemistry which would add a dimension to the Laboratory that is presently missing. This recruitment process is nearing completion.

The Laboratory has also initiated a search for a scientist to head the program in medical and molecular entomology. Medical entomology has always had a high priority in the LPD, but unfortunately it has been difficult to attract a leader with the unique blend of talents needed to match the high standards of the rest of the Laboratory. The field of medical entomology is relatively small and is just now undergoing a revitalization and reorientation, applying the tools of modern biology to the complex problems of disease vectors. This search process should be complete by the end of 1995.

An initiative has begun to develop a protein expression unit within David Kaslow's Malaria Vaccine Section. The need for such a unit is based on our experience that there is no facility in the worldwide malaria vaccine development effort which can produce the quantities of material needed for physical characterization and testing of immunogenicity in animal models. In

addition, the unit will be able to produce molecules such as those involved in host-binding, a phenomenon central to *Plasmodium falciparum* pathogenesis. Such molecules contain a cysteine-rich domain never before described. The unit could provide sufficient material for analysis of crystal structure, and for conducting assays to determine the ability of various constructs to block the binding interaction. Such studies could open the way for modern drug development through combinatorial chemistry. Another example is the 3' nucleotidase of *Leishmania* studied in Dennis Dwyer's laboratory. As this enzyme is not known to be expressed in mammalian systems, it has potential for use as a vaccine candidate, as a target for chemotherapy, or for immunodiagnostics. Production of this material in adequate quantities would permit full evaluation.

The Laboratory of Parasitic Diseases has purchased an atomic force microscope. Under the direction of Jim Dvorak this system may be able to approach atomic structure of living biological material. Its limits today are around 20 Å, and it is unknown if it will be able to overcome some of the physical limitations of the analytical tools. Dr. Dvorak has the theoretical background in biophysics, high resolution microscopy and computer technology to explore this potentially exciting new technique. He will spend three months in Japan working with one of the leaders in this new field. This system has the potential for becoming a major resource for the Institute.

The clinical parasitology program, previously headed by Eric Ottesen, has come under the leadership of Franklin Neva. The clinical unit will include Tom Nutman and Ted Nash. David Kaslow will become more involved because of his interests in vaccine research in humans.

RESEARCH PROGRESS

Dvorak Lab

The Biophysical Parasitology Program has succeeded in detecting subtle intra-specific differences in the genome of *Trypanosoma cruzi*, the causative agent of Chagas' disease. Analyses and characterization of these differences may help in our understanding of the presentation and course of disease. In addition, in collaboration with the Malaria group, the program has demonstrated important differences between the manner in which malaria-parasitized erythrocytes and gram negative bacteria are distributed within the spleen.

Dwyer Lab

1. 3'-Nucleotidase/nuclease:

a. To date, the gene encoding the unique, trypanosomatid surface membrane enzyme 3'-nucleotidase/nuclease (3'-NT/Nu) not identified from any source, including mammals, was isolated and characterized for the first time in *L. donovani* (*Ld*).

b. The previously observed nutrient-induced, over-expression of 3'-NT/Nu enzyme activity by *Ld* procyclic promastigotes was shown to be regulated at the mRNA level by transcriptionally mediated events.

c. A construct of the *Ld* 3'NT/Nu gene was transfected into *E. coli* and the resulting expressed protein possessed 3'-NT enzymatic activity.

2. Secretory acid phosphatases:

a. Three different *Ld* secretory acid phosphatase genes (SacP1, -2 and -3) were identified, characterized and their encoded proteins expressed in *E. coli*.

b. RT-PCR analyses demonstrated that mRNAs from all three SacP genes were constitutively transcribed in both *Ld* Pros and amastigotes.

c. Using light and fine structure immuno-cytochemistry, SacP was shown to be constitutively expressed by *Ld* Am's and to continuously traffick out of parasitophorus vacuoles into numerous secondary lysosomal vesicles which accumulate and fill the cytoplasm of infected macrophages.

3. Chitinase(s):

a. We identified a secretory chitinase from *Ld* promastigotes and characterized its biochemical properties.

b. The gene for this enzyme was isolated, characterized and expressed in *E. coli* as a 51 kDa protein. Transcripts of this chitinase gene were identified via RT-PCR using mRNA from *Ld* promastigotes.

4. Characterization of *Ld* developmental genes:

a. We have also identified fully sequenced and characterized several other *Ld* genes (i.e., an α -tubulin, two "stress-inducible" proteins: ST11 and gerC) that are either uniquely or differentially expressed by amastigotes, and these are being used as probes to study parasite gene-regulated differentiation and development.

b. Similarly, we have isolated and characterized a gene for calreticulin (i.e., a calcium-binding protein) in *Ld* and are investigating its expression and function.

5. Identification of genetic polymorphisms in *Leishmania*:

1. Finally, arbitrary, primed PCR methods were devised and used to identify both intra- and inter-specific gene polymorphisms in various *Leishmania* as well as to identify and characterize genetic loci which were either differentially or developmentally expressed in these parasites.

McCutchan Lab

We have developed auxotrophic lines of *P.falciparum* that are dependent upon exogenously supplied PABA for growth in culture. Biochemical analysis of these lines indicate that, unlike their wild type counterparts, they are unable to synthesize PABA. We have cloned these parasite

lines and have isolated the gene for PABA synthetase from wild type parasites. This is potentially significant at two levels. Historically, the development of auxotrophic lines of a microorganism has been an important step in gaining an understanding of the biochemistry of that organism. Further, immunological challenge of a host with auxotrophic lines of a pathogen has, in certain cases, triggered an effective immune response against the wild type pathogen.

We have defined the pattern of transcription of three distinctly different ribosomal RNA genes during the developmental cycle of the parasite. Molecular modeling of the three types of genes has pointed to fundamental differences in regions that are core to ribosome function. Experimental examination of these differences suggests that changes in ribosomal RNA expression patterns directly relates to the progression of parasite development.

Miller Lab

A. Receptor ligands in malaria

1. Last year we identified a motif involved in the binding of *P. vivax*, *P. knowlesi*, and *P. falciparum* erythrocyte binding molecules to receptors on erythrocytes. This motif, called region II, binds to multiple different receptors, depending on the ligand. The specificity of each parasite protein is determined by this region. Because a multicopy gene in *P. falciparum* had two-to-four copies of this motif (see *var* genes described from Wellems Lab), we showed that expression of this gene correlated with expression of the variant antigen on the erythrocyte surface. Importantly, the *var* gene expression also correlated with the pattern of ICAM-1 binding, suggesting that the variant antigen and the endothelial binding molecules are both on the same protein. It also suggests that the motif described for erythrocyte binding has also been used by the parasite for binding to endothelium. Variation in this motif gives the parasite great versatility for binding to different erythrocyte and endothelial receptors. Thus, this motif is central to pathogenicity in that it is involved in erythrocyte invasion, binding endothelium to escape parasite killing in the spleen, cerebral malaria, and antigenic variation.

2. The peptide responsible for binding of *P. vivax* and *P. knowlesi* to erythrocytes has been identified. The surprising finding that the rhesus peptide-blocked binding of *P. vivax* region II to human erythrocytes was surprising because *P. vivax* does not invade rhesus erythrocytes and *P. vivax* region II does not bind rhesus erythrocytes. The unusual finding was resolved by the demonstration that N-glycanase that removes N-linked sugars permitted binding of rhesus erythrocytes to *P. vivax* region II, demonstrating that the failure of rhesus erythrocytes to be invaded by *P. vivax* is because sugar groups block the peptide receptor.

B. Medical Entomology

1. Monoclonal antibodies have been successfully produced to the salivary glands and begin the biochemical description of this important gland in the parasite's life cycle in the mosquito.

2. Defensin, an immune peptide, kills parasites from the oocyst stage to sporozoites, but not ookinetes, the earliest form in the mosquito. This indicates a stage-specific change in the parasite's membrane. The other interesting finding was that defensin, like cercropin and magainin, are around 100 times more toxic to *Anopheles* than to *Aedes*.
3. The first step in the exploration of the midgut epithelial receptors was taken with the development of methods to study the interaction of ookinetes with midgut and the findings that invasion is not into epithelial cells, but into the so-called endocrine cells of the midgut.
4. Cryopreservation of *Anopheles* embryos has taken a major step forward in the development of methods to permeabilize *Anopheles gambiae* embryos without greatly reducing viability.

Nash Lab

1. Transient transfection system in *Giardia* has been developed.
2. Two *Giardia* cyst wall proteins have been identified, sequenced and the manner of transcription and secretion analyzed. Both proteins are not present in trophozoites but are induced in encysting organisms with identical kinetics. In contrast, Bip, a heat shock protein that is also increased during encystment, is controlled post transcriptionally. Therefore, control of proteins increased during encystment differ. Both cyst wall proteins combine to form a dimer and are further processed and secreted.
3. *Giardia* possesses the ability to modify proteins by isoprenylation and are inhibited or killed by compounds which inhibit this pathway.
4. Two genes present in the mitochondria of higher organisms have been found in *E. histolytica*. Since this organism lacks mitochondria, it suggests that *E. histolytica* as well as other protozoa which lack mitochondria may have had this organelle but later lost it. A different as yet unidentified residual organelle may be present in these organisms.
5. Monoclonal antibodies were produced to two species of microsporidia which has allowed identification of 3 of the major 4 species infecting humans to be identified.

Neva Lab

1. In collaboration with Drs. Nutman and Moore of LPD, and S. Williams of Smith College, a new and improved DNA library was prepared from rhabditiform and filariform larvae of *S. stercoralis* (Ramachandran and Neva).

2. Differences between L3's of *Strongyloides* and related nematodes have been demonstrated by analysis of PCR products of the internal spacer regions of ribosomal DNA genes (Ramachandran and Neva).
3. A clinical study of the association of *S. stercoralis* and HTLV-1 infection was initiated in collaboration with Dr. Edgar Carvalho in Brazil. Immune responses on patients with and without HTLV-1 are being examined with particular emphasis upon cytokine and immediate hypersensitivity skin test responses (Neva and Gam).
4. A double blind, placebo-controlled trial of topical therapy of the nonulcerating typical form of cutaneous leishmaniasis with paromomycin was carried out in Honduras. This form of treatment was not effective (Neva in collaboration with Dr. Carlos Ponce of Honduras).

Nutman Lab

1. Identified four novel Class II alleles (3 new DP alleles and 1 DQ allele).
2. Developed model system to induce B cell switching in vitro so that one can make antibody of both defined specificity and isotype.
3. Developed novel method for intracellular cytokine staining so that at the single cell level one can define using flow cytometry whether a given T cell is a Th1 or Th2 or Th0.
4. Identified both the correlates of immunity to both onchocerciasis and lymphatic filariasis and identified at least one possible vaccine candidate.
5. Created the cDNA libraries from both filariform and rhabditiform larvae of *S. stercoralis*.
6. Utilized both PCR and Circulating Antigen levels to demonstrate macrofilaricidal activity of DEC in bancroftian filariasis.
7. Demonstrated that there is a genetic underpinning to the resistance to infection with onchocerciasis (KM allotypes).

Sacks Lab

Developmental Biology of *Leishmania* Promastigotes

1. Stage-specific molecular and morphogenic markers were used in the first study of *Leishmania* metacyclogenesis within a natural phlebotomine vector. The appearance and positioning of infective stage parasites in the posterior midgut and foregut 7 to 10 days after infection was

associated with arabinose substitutions of sidechain oligosaccharides on the surface lipophosphoglycan, consistent with the selective egestion of unattached, metacyclic promastigotes during transmission by bite. Mutants defective in expression of specific sugars involved in midgut attachment have been characterized and the defective genes rescued by functional complementation.

2. Parasite isolates collected over the last 10 years from patients with visceral leishmaniasis in India were typed by monoclonal antibodies, isoenzymes, and kDNA analysis. The results confirmed that *L. tropica*, a species typically associated with self-limiting cutaneous disease, is a coendemic agent of kala-azar in India and may shed light on the rising frequency of antimony unresponsiveness which complicates treatment of this lethal disease. Thus, a major disease in India is proven to be caused by *L. tropica* as well as *L. donovani*.

Sher Lab

A. Schistosomiasis

1. Demonstration that IL-12 can be used as the basis for a vaccine for preventing egg pathology (granuloma formation and fibrosis) due to natural schistosome infection. (Previous work was in artificial egg injection model). First functional antipathology vaccine for a parasitic disease.

2. Demonstration that IL-12 can also be used as an adjuvant to enhance protective immunity induced by an attenuated (irradiated cercariae) vaccine.

3. Identification of calpain (a Ca^{++} -dependent neutral protease) as the target antigen of a protective T cell clone by library screening and gene sequencing. First schistosome vaccine candidate identified by its reaction with T cells.

4. Use of knockout mice to demonstrate lack of requirement of B cells and $\text{Fc}\epsilon\text{R}^{+}$ non B, non-T cells (Bill Paul "basophil like" IL-4-producing cells) in development of Th2 response in schisto infected mice.

5. First in vivo demonstration of a novel IL-4-response pathway involving antigenic stimulation of CD4^{+} cells leading to IL-3 production which in turn stimulates IL-4 secretion by $\text{Fc}\epsilon\text{R}^{+}$ non-B, non-T cells.

B. Intracellular opportunistic infections

1. Demonstration of defective parasite-specific IFN- γ and IL-12 responses in HIV-positive, *Toxoplasma gondii*-positive patients. First demonstration that IL-12 responses to an endogenous infection (as opposed to in vitro mitogen) are suppressed during HIV infection.

2. Demonstration that superantigen reactive V β 5+ T cells are anergized as infection shifts from the acute to the chronic stage. (First demonstration of such phenomenon in parasitic infection.)
3. Demonstration that both IFN- γ and IL-10 knockout mice are highly susceptible to normally avirulent *T. gondii* infection. The latter observation is particularly interesting since mice die of "shock" due to excessive cytokine (TNF- α , IL-12 IFN- γ) response rather than uncontrolled parasite growth suggests that IL-10 is induced to downregulate excessive host cytokine response.
4. Demonstration that monokine (IL-12, TNF- α , IL1 β and IL-10)-inducing activities in *T. gondii* are glycoconjugates which fall into two groups in terms of their biochemistry and the PKC-dependence of their induction pathways.

Ward Lab

1. Using electrophysiology techniques, Ward has shown that *Toxoplasma gondii* invades the host cell by invaginating the host membrane, not by creating its own membrane from rhoptry contents as was thought by others. In these same studies, an electrical spike was observed when the parasite came into contact with the host cell. This begins the study of the nonreceptor events in invasion of Apicomplexa into host cells.
2. Some of the components of the *Plasmodium* spp. and *T. gondii* parasite's proteins involved in the invasion machinery have been identified on actin-binding columns.

HONORS AND AWARDS

Chetan Chitnis received the Young Investigator Award of the American Society of Microbiology.

James Dvorak received a Distinguished Scientist Fellowship from the Japan Society of the Promotion of Science and will spend a three-month sabbatical in Japan.

Dennis Dwyer serves on the editorial boards of *Experimental Parasitology*, *Journal of Eukaryotic Microbiology* and *Tropical Medicine Abstracts*.

David Kaslow has been named to the editorial board of *Experimental Parasitology* and serves on the Medical Research Oversight Committee of the Office of Naval Research.

Thomas McCutchan has been appointed to the Editorial Board of *Experimental Parasitology*. He has also served on the Editorial Board of *Molecular and Biochemical Parasitology*. A Sloan Fellowship was awarded to study the molecular evolution of malaria parasites by the National Science Foundation.

Louis Miller presented the Award Lecture of the American Association of Blood Banks in November 1994; was organizer of the International Conference on Transformation of Invertebrates in Montpellier, France, April 22-26, 1995; was organizer of the International Meeting on Interaction of *Anopheles* and Malaria at Les Treilles, France, September 16-22, 1995; presented the Lynch Lectures at the University of Notre Dame October 5-6, 1995, and was a Grollman Visiting Professor at the University of Maryland, October 18, 1995.

Franklin Neva was a Scientific Consultant for the Malaria Vaccine Development Program of the U.S. Agency for International Development, 1994 to present; was a member of Ethical Review Committee on Clinical Studies for Pan American Health Organization, 1990 to present; was a member of the Editorial Boards of the following journals: a) *The Journal of Infectious Diseases*, 1990 to present; *The Journal of Antibiotics and Chemotherapy*, 1988 to present; and the International Advisory Board of the *Memorias do Instituto Oswaldo Cruz* in Brazil, 1993 to present. He served as Acting Scientific Director, Division of Intramural Research, NIAID, May 1, 1994 - June 24, 1995, and was Director, Intramural Center for Tropical Disease Research, NIAID.

Thomas Nutman has been named an editor of the *American Journal of Tropical Medicine and Hygiene*, a section editor of the *Journal of Immunology*; he has been appointed to the Clinical Tropical Medicine Certification Examination Board.

David Sacks received the NIH Director's Award in 1995, serves as Chairman of the TDR/WHO Leishmaniasis Steering Committee, and was co-organizer of a meeting on "Second Generation Vaccines Against Leishmaniasis," Salvador, Brazil.

Alan Sher served as coeditor of a special issue on microbial immunity in *Current Opinion in Immunology* and was coorganizer of a "Conference on Interleukin-12 in Infection: Prospects for Prophylactic and Therapeutic Intervention." He was an invited speaker at the Biology of Parasitism Course at Woods Hole, Massachusetts; the Max Planck Institute for Immunology in Freiburg, Germany; the Wellcome Trust, London, England; and the American Association of Immunologists in Atlanta, Georgia.

Thomas Wellems received the NIH Director's Award and serves on the Editorial Board for *Molecular and Biochemical Parasitology*.

AIDS-Related Projects within the LPD:

<u>Section</u>	<u>Percent effort on AIDS</u>
Immunobiology (Sher)	45%
Cell Biology (Dwyer)	100%
Gastrointestinal Parasites (Nash)	20%
Intracellular Parasite Biology (Sacks)	50%
Clinical Parasitology (Neva)	100%

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00094-35 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Entamoeba histolytica: Cellular Physiology and Molecular Diagnostics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	T.E. Nash	Section Head	LPD, NIAID
Others:	C.G. Clark	Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasite Growth and Differentiation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED 10/1/94 (covered under Z01 AI00161-18 and Z01 AI 00350-13)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00099-25 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Dvorak	Res. Microbiologist	LPD, NIAID
Others:	Carol C. Cunnick	Biologist	LPD, NIAID
	D. B. Rainey	Biological Lab. Tech.	LPD, NIAID
	C. P. Mudd	Senior Engineer	ACE, BEIP
	S.E. Abdallah	Guest Worker	LPD, NIAID
	David W. Alling	Special Assist. Biometry	OD, NIAID
	Stefan Karlsson	Chief, Mol. Genetics	NINDS
	Anjali Yadava	Visiting Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Program, DRR; Molecular and Medical Genetics Section, NINDS; Office of the Scientific Director, NIAID; Keio University, Tokyo

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Biochemical and Biophysical Parasitology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

1.0

OTHER:

3.0

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- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with analyses of the genetic diversity of medically important parasitic protozoa and its implications to the epidemiology, course, and diagnosis of disease. The project has become increasingly involved in elucidation of diversity at the molecular level. Due to circumstances beyond our control, we have terminated several ongoing projects described in previous reports and have concentrated our efforts in three areas: (1) the detection and elucidation of intra-specific differences in *Trypanosoma cruzi*. Using the RAPD assay we have identified an oligonucleotide which differentiates between stocks of *T. cruzi* at the DNA level. Two unique DNAs of 279 and 290 bp have been isolated and are partially characterized. (2) We are attempting to apply the mathematical modeling methods we developed for analyses of the DNA synthetic cycle of *Entamoeba* spp. to *Giardia lamblia*. The method involves the use of a computer model to decompose flow cytometer-derived DNA histogram into their G1, S, and G2 components. (3) We have developed low-light-level video microscopy methods usable for studies of the role of the spleen in a malaria infection. Even though the spleen is known to be a site of parasite clearance, the mode of action is not known. We use a fluorescent lipophilic dye to mark the membrane of parasitized erythrocytes and a fluorescent monoclonal antibody to mark the marginal macrophages of the spleen. Images of each reaction are captured into a computer and merged electronically to evaluate the role of the spleen in a malaria infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00102-21 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.A. Neva	Head, Unit on Clinical Parasitology	LPD, NIAID
	D. Sacks	Research Microbiologist	LPD, NIAID
	A. Gam	Biol. Lab Tech. (Micro.)	LPD, NIAID
	R. Kenney	Sr. Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Dept. Biology, Youngstown State Univ. (R. Kreutzer); Ministry of Health, Tegucigalpa, Honduras (C. Ponce); Walter Reed Army Med. Center (A. Magill and C. Oster); Institute of Medical Sciences, Banaras Hindu University, Varanasi, India (S. Sundar)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit on Clinical Parasitology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses upon the different clinical forms of leishmanial infections in humans, immune responses to the parasite with particular references to cell-mediated response and the cytokine regulation of these responses, and characteristics of the causative parasites.

Ten patients were referred to NIH for suspected leishmaniasis. Seven were U.S. Peace Corps volunteers or tourists, and two were military personnel, one of whom had been in the Persian Gulf region and was suspected of having visceralizing leishmaniasis. The other military case had proven visceral leishmaniasis acquired in southern Spain. One patient was a foreign service officer with an undiagnosed illness. Seven of these patients were treated with Pentostam (sodium stibogluconate) at the Clinical Center. The double-blind, placebo-controlled trial of topical treatment with paromomycin of atypical, nonulcerating cutaneous leishmaniasis in Honduras was completed. A total of 53 patients were enrolled in the trial, but the topical paromomycin as clearly not effective. One surprising outcome of the study was the finding that more than half of the 26 cases from one rural locality were caused by *L. mexicana* species rather than exclusively due to *L.d. chagasi*, as had been found previously.

Collaborative studies with Dr. Sundar at Banares Hindu University in India were initiated on patients with visceral leishmaniasis. The object of the studies is to examine the cytokine response of bone marrow in patients with active visceral disease. Additional isolates of parasites from kala azar cases are also desired since *L. tropica* species instead of the expected *L. donovani* was recently found as the cause of disease in some instances.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00108-24 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Kaslow	Section Head	LPD, NIAID
Others:	S. Kumar	Staff Fellow	LPD, NIAID
	L.H. Miller	Chief	LPD, NIAID
	T. Jing-Hui	Visiting Fellow	LPD, NIAID
	A. Yadava	Visiting Fellow	LPD, NIAID
	D.B. Keister	Biologist	LPD, NIAID

COOPERATING UNITS (if any)

K. Perdue, Veterinary Resources Branch, NIH; J.A. Bersofsky, Molecular Vaccine Branch, NCI; A. Agar, U. of Miami; W.E. Collins, CDC, Atlanta, GA; G. Milon, Institut Pasteur, Paris, France; E. Classen, Holland: W. Jacobs and B. Bloom, Albert Einstein Univ., New York; B. Wellde, WRAIR, Washington, DC

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892-0425

TOTAL STAFF YEARS:

3.7

PROFESSIONAL:

3.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunity that provides protection against the morbidity and mortality of malaria remains poorly understood. Likewise, the immune responses that mediate protective immunity to subunit vaccination in animal models is also presently unclear. In the absence of reliable in vitro correlates of protective immunity and with the lack of a full understanding of what contributes to immunity to malaria, development of effective malaria vaccines will continue to be haphazard at best. The major goals of this project are to elicit and then define protective immunity against malaria in animal models. We have now elicited protective immunity by subunit vaccination with the major surface protein, MSP1, in both rodent (*P. yoelii* in mice) and primate (*P. falciparum* in *Aotus* monkeys) models. Although immunity in these model systems may be antibody-dependent, it appears that antibodies alone, as measured by ELISA, do not mediate protective immunity. A variety of in vitro assays have been undertaken to search for the mechanisms of this subunit vaccine-induced protective immunity. Vaccination with whole malaria parasites and *Salmonella* elicits protective immunity to *P. vinckei* in mice. The immunity in this animal model system is primarily cellular-mediated, CD4+ T cell-dependent and requires the spleen. We are using this model to study the contribution of the spleen in providing protective immunity and, in conjunction with recombinant *Salmonella* and BCG, to identify malaria parasite polypeptides (T cell epitopes) that lead to protection. As of January 1996, this project will terminate and any ongoing and future studies will be incorporated into the Recombinant Protein Expression Unit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00161-18 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: T.E. Nash Medical Officer LPD, NIAID
 Others: M.M. Mowatt, Staff Fellow, LPD, NIAID; D.H. Luján, Visiting Fellow, LPD, NIAID; L. Byrd, IRTA, LPD, NIAID; A. Sher, LPD, NIAID; M. Chance, Asst. Professor, Albert Einstein College of Med.; R. Leapman, Biomedical Engineer, NCRR; L. Helman, Pediatrics Branch, NCI; B. Gottstein, Institute of Parasitology, University of Zurich, Switzerland; D. Darwood, NIAID Rocky Mountain Laboratories; B. Bowers, Laboratory of Cell Biology, NHLBI, NIH; Lippincott-Schwartz, Cell Biology & Metabolism Branch, NICHD, NIH; Martha Espinosa, Centro de Investigación y de Estudios Avanzados del I.P.N., México City, México. Guest Worker: H.H. Stibbs, Dept. of Cell & Molecular Biology, Tulane Univ., New Orleans, LA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During encystment, *Giardia* undergoes fundamental biological changes. We identified two unique cyst wall proteins, cwp1 and cwp2, as well as the ER resident chaperonin, Bip, which allowed us to study how the level and amount of these proteins are controlled, the nature of the vesicular transport of cwp 1 & 2 into encystment-specific vesicles, and the formation of the cyst wall. Cwp2 was identified, characterized and sequenced. Studies showed that it is secreted similar to cwp1 and likely forms a stable complex with cwp1. Bip expression increases markedly during encystment; however, in contrast to cwp1 and 2, control appears posttranscriptionally. The first description of protein isoprenylation in any protozoan was described in *Giardia*. This indicates posttranslational modification by farnesyl, and geranylgeranyl isoprenoids occurs in *Giardia* and is important in the regulation of growth of this primitive eukaryote. A panel of monoclonal antibodies were produced which recognize three of the four major species of microsporidia which infect humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00162-19 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD, NIAID
Others:	A. Debrabant	Visiting Fellow, FIC	LPD, NIAID
	S.L. Ellis	Biologist	LPD, NIAID
	P.F.P. Pimenta	Guest Investigator	LPD, NIAID
	D.L. Sacks	Supervisory Microbiologist	LPD, NIAID
	A.M. Shakarian	IRTA Fellow	LPD, NIAID

COOPERATING UNITS (if any)

CBER, DHP, FDA (M. Joshi, N.S. Lee, G.P. Pogue & H. L. Nakhasi); DMID, NIAID (M. Gottlieb); London School Tropical Med.& Hyg. (P. Kaye).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-0425

TOTAL STAFF YEARS:

5.5

PROFESSIONAL:

4.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cell and molecular biology of the human pathogen, *Leishmania*, is investigated as a model of both intra- and extracellular parasitism. Emphasis is placed on characterizing both the biochemical functions and gene structure of their surface membrane and secreted proteins toward defining roles for these constituents in parasite survival and development.

The gene encoding the unique, trypanosomatid surface membrane enzyme 3'-nucleotidase/nuclease (3'-NT/Nu) was isolated and characterized from *L. donovani* (*Ld*). The nutrient-induced over-expression of *Ld* 3'-NT/Nu enzyme activity was shown to be regulated at the mRNA level by transcriptionally-mediated events. A construct of this gene was transfected into *E. coli* and the resulting expressed protein possessed 3'-NT enzymatic activity. In other studies, three different *Ld* secretory acid phosphatase genes (SACP1, -2 and -3) were identified, characterized and their encoded proteins expressed in *E. coli*. RT-PCR analyses demonstrated that mRNAs from all three SACP genes were constitutively transcribed in both *Ld* procyclic promastigotes (Pros) and amastigotes (Am). Using immuno-cytochemistry, SACP was shown to be constitutively expressed by *Ld* Am's and to continuously traffick out of parasitophorus vacuoles into numerous secondary lysosomal vesicles which accumulate and fill the cytoplasm of infected macrophages. In other studies, we identified a secretory chitinase from *Ld* Pros and characterized its biochemical properties. Further, the gene for this enzyme was isolated, characterized and expressed in *E. coli* as a 51 kDa protein. Transcripts of this chitinase gene were identified via RT-PCR using mRNA from *Ld* Pros. We have also identified and characterized several other *Ld* genes which are either uniquely or differentially expressed by Am's and these are being used as probes to study parasite gene-regulated differentiation and development. Finally, arbitrary primed PCR methods were devised and used to identify both intra- and inter-specific gene polymorphisms in various *Leishmania* as well as to identify and characterize genetic loci which were either differentially or developmentally expressed in these parasites.

The current results are of relevance toward identifying targets for the development of new diagnostic, chemotherapeutic and immunoprophylactic agents against these important human pathogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00197-16 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation and Immune Recognition in Filariasis and Non-filarial Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	T.B. Nutman	Section Head	LPD, NIAID
Others:	S. Mahanty	Senior Staff Fellow	LPD, NIAID
	L. Elson	Visiting Fellow, FIC	LPD, NIAID
	P. Zimmerman	NRC Fellow	LPD, NIAID
	S. Mawhorter	Clinical Associate	LPD, NIAID
	C. Prussin	Clinical Associate	LCI, NIAID
	C. Steel	Biologist	LPD, NIAID
	J. McCarthy	Visiting Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Indian Council of Medical Research, Madras, India (V Kumaraswami); Anna University, Madras, India (K Jayaraman); CPqAM/FIOCRUZ, Health Department, (Cook Islands, A. Guinea); Hospital Vozandes, Quito, Ecuador (R. Guderian); Onchocerciasis Chemotherapy Centre, Hohoe, Ghana (K. Awadzi)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Helminth Immunology Section

INSTITUTE AND LOCATION

NIAID, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.7

PROFESSIONAL:

4.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(Cooperating Units Cont.) - Univ. of Manchester, Manchester, U.K. (J. Bedlam)

The purpose of this project is to delineate the mechanisms involved in regulating immune responses in filarial and nonfilarial disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy to be a result of the production of the antiproliferative cytokine, IL-10, made by both monocytes and CD4+ cells (of the Th2 type). The phenotypic characterization of these Th2 CD4+ cells has shown them to be CD45RO+CD27-; a novel method for intracellular staining for cytokines has shown that IL-5 and interferon gamma are the best discriminators of Th2 and Th1 T-cell subsets. Using selected recombinant filarial antigens, the role the antigens themselves play in the induction of a Th2 response and the B cell response it subsequently influences (IgE/IgG4) has been studied. Similarly, new ways of assessing eosinophil activation have also been developed.

Clinical epidemiological and parasitological assessment of individuals who are "putatively immune" to either onchocerciasis or lymphatic filariasis have been identified. These individuals very clearly mount augmented cellular responses to parasite antigens and, for those immune to onchocerciasis, responses to a recombinant antigen (OV20/11) have been associated with resistance, and suggests a mechanism for protection.

The genetics underlying susceptibility and resistance to filarial infection has been studied by HLA Class II typing along with allotyping. A particular KM allotype has been identified as correlating with resistance to infection. Also, a number of novel DQ and DP alleles have been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00208-15 LPD
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT The Isolation and Characterization of Plasmodial Genes		
PRINCIPAL INVESTIGATOR	Thomas F. McCutchan	Microbiologist LPD, NIAID
Others:	G. McConkey	Senior Staff Fellow LPD, NIAID
	M. Sullivan	Technician LPD, NIAID
	Jun Li	Guest Worker LPD, NIAID
	M. J. Rogers	Staff Fellow LPD, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Growth and Development Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) We have defined the pattern of transcription of the developmentally regulated rRNA genes of <i>Plasmodium</i> species. We have found that upregulation of one of the genes relates to the differentiation of the sporozoite as a unit within the oocyst. We have also discovered a previously undescribed rDNA unit that is expressed only during oocyst development in the mosquito abdomen, but never carried to the thorax in mature sporozoites. Molecular modeling of the oocyst gene reveals that it would suppress UGA termination; hence, its presence in the translation apparatus would result in fundamental changes in the pattern of translation products that occur during development. We consider the elucidation of the characteristics of this switch of importance with regard to understanding translational control in the parasite. To more directly study the ribosomal rRNAs, we have studied the susceptibility of parasites to both cytoplasmic and organellar ribosome inhibitory drugs. We have shown characteristically different effects of the drugs. We have also produced resistant mutants to one of the cytoplasmic ribosome inhibitors, anisomycin. In separate studies, we have continued our work with attenuated lines of human and rodent malaras. Auxotrophic lines of parasites have been cloned and their developmental characteristics studied. Auxotrophic mutants of both species of parasite have been defined by their dependency on either exogenous pyrimidines or para-aminobenzoic acid. These should prove useful in the study of both the biochemistry of parasites and the development of immunity to parasites.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00240-14 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.C. Kaslow, Section Head, LPD, NIAID

Others: M. Shahabuddin, Visiting Associate, LPD, NIAID; M. Fried, Visiting Fellow, LPD, NIAID; T. Templeton, IRTA Fellow, LPD, NIAID; P. Duffy, Guest Researcher, LPD, NIAID; M. Gozar, Visiting Fellow, LPD, NIAID; D. Miller, IRTA Fellow, LPD, NIAID; J. Vinetz, IRTA Fellow, LPD, NIAID; D. Keister, LPD, NIAID; O. Muratova, Special Volunteer, LPD, NIAID; R. Hearn, Entomologist, LPD, NIAID; B. Wood, Pre-IRTA Fellow, LPD, NIAID; P. Moorthy, Pre-IRTA Fellow, LPD, NIAID; K. Land, Summer Fellow, LPD, NIAID

COOPERATING UNITS (if any)

(see attached)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Vaccine Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

8.6

PROFESSIONAL:

6.3

OTHER:

2.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Over two million children in Africa will die of malaria this year. To control malaria, several vaccine approaches are being developed, one of which is against the sexual stages (aka a transmission-blocking vaccine). The genes encoding four potential transmission-blocking target antigens (Pfs25, Pfs28, Pfs40, and Pfs230) of transmission-blocking antibodies have now been cloned in our laboratory, and a fifth, a parasite-produced chitinase, and a sixth, a mosquito-produced protease, have been identified. Of the four target antigens that have been cloned, all have been expressed in one or more recombinant expression systems. rPfs25, rPfs28 and rPfs230 have induced transmission-blocking antibodies in laboratory animals. Our immediate goals are to 1) test in humans the safety, immunogenicity, and efficacy of a rPfs25 subunit vaccine and design a means of testing the efficacy of such a transmission-blocking vaccine in the field, 2) improve expression of the rPfs28 that induces blocking antibodies and test various combinations of rPfs25 and rPfs28 in a cocktail vaccine suitable for use in humans to determine if the combination elicits longer lasting or higher titer transmission-blocking antibodies, 3) determine the role, if any, that the calcium-binding Pfs40 plays in sexual development and ascertain if Pfs40 is a target of transmission-blocking antibodies, 4) improve the expression of rPfs230 so that it induces antibodies equivalent to those of transmission-blocking mAbs to Pfs230, 5) isolate and express the genes encoding the parasite-produced chitinase and mosquito-produced proteases, and 6) isolate analogous genes to the five parasite proteins, if they exist, from *P. vivax*. Our more long-term goals include identifying new target antigens on sexual stage parasites, and defining the molecular mechanisms involved in fertilization of malarial parasites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00241-14 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT

Receptors for Invasion of Host Cells and Adhesion to Endothelium

PRINCIPAL INVESTIGATOR

L. H. Miller

Chief

LPD, NIAID

Others:

D. Hudson-Taylor

Microbiologist

LPD, NIAID

C. Chitnis

Visiting Fellow

LPD, NIAID

J. Smith

IRTA

LPD, NIAID

G. Ward

Visiting Associate

LPD, NIAID

I. Tardieux

Visiting Associate

LPD, NIAID

COOPERATING UNITS (if any) C. Newbold, A. Craig, and D. Roberts, Oxford University, UK; T. Hadley and Stephen Pifer, University of Louisville; KL Sim, Entremed; O. Pogo and A. Chauduri, New York Blood Center; JoLynn Proctor, NIH Blood Center; B. Faulkner, Pennsylvania Medical

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.5

PROFESSIONAL:

5.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

(Cooperating units cont.)

School; E. Sass-Toby, NICHD; G. Langsley, Institute Pasteur, Paris; Y. Raviv, NIDDK.

The merozoite and the infected erythrocyte interact in a receptor-specific manner with erythrocyte and endothelium, respectively, to promote invasion of erythrocytes and cytoadherence to endothelium. These steps are critical in the pathogenesis of malaria and are targets for vaccines and drugs to block invasion and cytoadherence. We are identifying the *P. knowlesi*, *P. vivax* and *P. falciparum* parasite ligands and the host receptors for invasion and the molecular basis of antigenic variation. An antigen on the erythrocyte surface involved in antigenic variation and cytoadherence has been the focus of study because of its importance to pathogenesis, including its having the same motif as the parasite molecule involved in erythrocyte invasion. The components in the junction and the signaling after malaria merozoites and *Toxoplasma gondii* make contact with their host cells is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00248-14 LPD						
PERIOD COVERED October 1, 1994 to September 30, 1995								
TITLE OF PROJECT Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes								
PRINCIPAL INVESTIGATOR Others:	<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">L. H. Miller</td> <td style="width: 33%;">Laboratory Chief</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>M. Shahabuddin</td> <td>Visiting Associate</td> <td>LPD, NIAID</td> </tr> </table>		L. H. Miller	Laboratory Chief	LPD, NIAID	M. Shahabuddin	Visiting Associate	LPD, NIAID
L. H. Miller	Laboratory Chief	LPD, NIAID						
M. Shahabuddin	Visiting Associate	LPD, NIAID						
E. Mialhe Guest Researcher LPD, NIAID E. Saravia Visiting Fellow LPD, NIAID D. Seeley Supervisory Entomologist LPD, NIAID P. Pimenta Guest Researcher LPD, NIAID K. D. Vernick Senior Staff Fellow LPD, NIAID R. W. Gwadz Assistant Chief LPD, NIAID	D. C. Kaslow Head, Malaria Vaccine Sec., LPD, NIAID C. Barreau Visiting Fellow LPD, NIAID A. Laughinghouse Research Entomologist LPD, NIAID G. C. Lanzaro Senior Staff Fellow LPD, NIAID H. Zieler IRTA Fellow LPD, NIAID M. P. Valencia Visiting Fellow LPD, NIAID							
COOPERATING UNITS (if any) National School of Medicine, Bamako, Mali (Dr. Y. Toure); Oak Ridge National Laboratory (Dr. P. Mazur); Case Western Reserve University (Drs. H. Fujioka and M. Jacobs-Lorena); Biological Laboratories, The European Molecular Biology Laboratory, Heidelberg, Germany								
LAB/BRANCH Laboratory of Parasitic Diseases								
SECTION Medical Entomology Section								
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892								
TOTAL MAN-YEARS: 9.2	PROFESSIONAL: 5.8	OTHER: 3.4						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews								
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) (Cooperating Units Cont.) (Drs. F. Kafatos and B. Zheng); Department of Molecular Biology and Biochemistry, University of California-Irvine (Dr. A. James). The biology of <u>anopheline mosquitoes</u> is being studied in relation to the capacity of these vectors to transmit <u>malaria</u> . <u>Genetic, molecular, biochemical, and immunological</u> studies are seeking to describe mechanisms in the mosquito that enhance or retard development of the malaria parasite with emphasis on the behavior of <u>ookinetes, oocysts, and sporozoites</u> . To facilitate these studies: 1) methods are being improved for <u>cloning and transposing genes</u> into <u>mosquito germ lines</u> , and 2) systems are being evaluated for the <u>cryopreservation</u> of mosquito embryos to increase our capacity to store genetic material. The ability to identify, clone, and transpose genes that regulate mechanisms that render mosquitoes refractory to parasite development should facilitate development of control strategies based on the introduction into the field of mosquito populations incapable of transmitting malaria.								

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00251-14 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	T. Wynn	IRTA Fellow	LPD, NIAID
	D. Jankovic	IRTA Fellow	LPD, NIAID
	A.W. Cheever	Senior Investigator	LPD, NIAID
	M. Kullberg	Special Volunteer	LPD, NIAID
	L. Aslund	Special Volunteer	LPD, NIAID
	S. Fouad	Special Volunteer	LPD, NIAID
	S. James	Chief	PTDB, MIDR, NIAID

COOPERATING UNITS (if any)

MET, NCI, Bethesda, MD (J. Berzofsky); Biomedical Research Institute, Rockville, MD (F. Lewis); Univ. of Kentucky, Louisville, KY (F. Botran)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

4.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The aim of this research program is to investigate mechanisms of protective immunity and immunopathology in schistosomiasis with the ultimate goal of immunologic intervention. Progress was achieved in the following areas during the year.

A. Prophylaxis of egg pathology with an IL-12-based vaccine. Immunization of mice with egg antigens plus IL-12 was shown to block egg pathology resulting from subsequent schistosome infection. In a related project, IFN- γ knockout mice were used to analyze the IFN- γ dependence of the suppressive effects of IL-12 on egg pathology.

B. Identification of calpain as a candidate vaccine antigen. The antigen recognized by a protective T cell clone was identified by screening a recombinant expression library and was shown to be a worm neutral protease, calpain.

C. Mechanisms of IL-4 induction in murine schistosomiasis. Using gene knockout mice, the induction of IL-4 by schistosome infection was shown to be independent of B cells and Fc gamma and epsilon receptors on non-B, non-T (NBNT), IL-4-producing cells. In related work a new mechanism of IL-4 induction was identified which involves the triggering of cytokine production from NBNT cells by IL-3 from antigen stimulated CD4+ cells. Finally, the expression of soluble IL-4 receptor was shown to be linked to IL-4 synthesis during murine schistosome infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00253-14 LPD

October 1, 1994 to September 30, 1995

Studies of Immunological Responses to Filarial Infections

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	C. Steel	Biologist	LPD, NIAID
	S. Mahanty	Medical Staff Fellow	LPD, NIAID
	J. McCarthy	Visiting Fellow	LPD, NIAID
	P. Zimmerman	NRC Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Indian Council of Medical Research, Madras, India (S. Tripathy, V. Kumaraswami); Anna University, Madras, India (K. Jayaraman); CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Coutinho); Health Department, Cook Islands (A. Guinea); (Cont below)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology

INSTITUTE AND LOCATION

NIAID,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(Cooperating Units Cont.)

James Cook University, Queensland, Australia (P. Turner); Washington University, St. Louis, MO (G. Weil); Smith College, Northhampton, MA (S. A. Williams)

TERMINATED (being absorbed by Project Z01 AI 00197-16)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00256-14 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Biology of *Leishmania* Promastigotes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Other:	P. Pimenta	Visiting Associate	LPD, NIAID
	R. Kenney	Senior Research Investigator	LPD, NIAID
	G. Modi	Visiting Fellow	LPD, NIAID
	B. Butcher	IRTA	LPD, NIAID

COOPERATING UNITS (if any)

Dr. Sam Turco, Dept. of Biochemistry, University of Kentucky; Dr. Stephen Beverley, Harvard Medical School, Boston

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Intracellular Parasite Biology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stage-specific molecular and morphogenic markers were used to follow the kinetics of appearance, number, and position of metacyclic promastigotes developing during the course of *L. major* infection in a natural vector, *Phlebotomus papatasi*. Expression of surface lipophosphoglycan (LPG) on transformed promastigotes was delayed until day 3, and continued to be abundantly expressed by all promastigotes thereafter. An epitope associated with arabinose substitution of LPG side-chain oligosaccharides was detected on the surface of a low proportion of midgut promastigotes beginning on day five, and on up to 60% of promastigotes on days 10 and 15. In contrast, 100% of the parasites egested from the mouthparts during forced feeding of 15-day infected flies stained strongly for this epitope. A metacyclic-associated transcript (MAT-1) was used in *in situ* hybridization studies to demonstrate the positioning of metacyclics in the anterior gut.

L. major mutants defective in the expression of side-chain sugars on LPG, which are involved in midgut adhesion, were obtained by negative selection using lectins and antibodies. The absence of specific sidechain sugars was shown by *in vitro* biosynthetic studies using the phosphoglycan backbone as acceptor to be due to the lack of a galactosyl transferase. Attempts to clone the transferase by functional complementation of the defective gene have been frustrated by the high frequency of chromosomal integration of the cosmid in the rescued clones. The cosmid DNA has recently been recovered by restriction digests of total DNA from the rescued phenotypes, re-ligation into the cosmid vector for bacterial transformation and cloning.

The importance of the sand fly peritrophic matrix to the transformation, growth and development of *Leishmania major* in *P. papatasi* was investigated. Sand flies were infected by membrane feeding on amastigotes containing whole blood, with or without added chitinase. Transformation to and growth of promastigotes were observed in dissected midguts from all control flies by 36 hrs, whereas no viable promastigotes were observed in midguts from the chitinase-treated flies. When chitinase-treated flies were infected with logarithmic phase promastigotes instead of amastigotes, viable midgut infections developed. These data suggest an essential role for the peritrophic matrix in protecting the parasite during its early stage of development from the potentially lethal activities of the bloodfed midgut.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00257-14 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.A. Neva	Head, Clinical Parasitology Unit	LPD, NIAID
Others:	A. Gam	Technician	LPD, NIAID
	S. Ramachandran	Visiting Fellow	LPD, NIAID
	T. Nutman	Head, Clinical Parasitology Sect.	LPD, NIAID
	T. Nash	Head, Host Parasite Relations Sect.	LPD, NIAID

COOPERATING UNITS (if any)

Federal University of Bahia, Brazil (E. Carvalho and A. and M. Barral); Univ. of Penn. School of Veterinary Medicine (G. Schad); Univ. of West Indies, Jamaica (R. Robinson)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

0.9

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project deals with both the laboratory and clinical aspects of infection caused by the intestinal nematode, *Strongyloides stercoralis*. The laboratory research involves analysis and characterization of parasite antigens. The clinical studies focus upon the immune response of infected individuals, and factors that influence the immune response. Experimental infections with the parasite in a newly recognized animal host, the jird (*Meriones unguiculatus*), have been initiated recently. Also, collaborative clinical studies are being carried out in Brazil.

The recombinant gene product (S4) reported last year could not be cleanly separated from its pMAL vector so a new cDNA library was prepared. Evidence for differences between L3s of strongyloides and related nematodes are being examined by analysis of PCR products of internal spacer regions of ribosomal DNA genes.

Analysis of cytokine responses in 17 Brazilian patients with *S. stercoralis* infections, with or without concomitant HTLV-1 infection, have disclosed elevated INF-gamma responses in those with HTLV-1. Immediate skin tests are being done on these and additional patients. In preliminary studies it appears that IgG4 antibodies decline much earlier than total IgG antibodies in treated strongyloidiasis patients.

Strongyloides infections of jirds are not easily maintained in serial passage, but this experimental host may be useful for certain studies that are impractical in dogs or monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-13 LPD

PERIOD COVERED

October 1, 1994 - July 1, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.W. Cheever	Head, Host-Parasite Relations	LPD, NIAID
Others:	G. Yap	Visiting Fellow	LPD, NIAID
	R. Poindexter	Bio. Lab. Tech.	LPD, NIAID
	T. Wynn	Immunology Sect.	LPD, NIAID
	A. Sher	Head, Immunology Sect.	LPD, NIAID

COOPERATING UNITS (if any)

Department of Medicine, USUHS (Fred Finkelman) and USDA (Joseph Urban); Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade); Department of Microbiology, New York State College of Veterinary Medicine, Ithaca, NY (Dr. Edward Pearce)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

1.6

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Morbidity in schistosome-infected individuals is caused mainly by the immune response of the host to schistosome eggs deposited in the tissues. In chronic schistosomiasis, fibrotic sequelae to the inflammatory reaction to the eggs are responsible for most clinical disease.

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Cytokines play an important role in the genesis and regulation of the size of circumoval granulomas and in the fibrosis associated with them.

Anti-IL-4 treatment of *S. mansoni* or *S. japonicum*-infected mice resulted in a slight decrease in granuloma size, but a major decrease in hepatic fibrosis and a considerable decrease in Th2-type cytokine secretion (IL-4 and IL-5) and an increase in Th1-type cytokines (IFN- γ and IL-2). Cytokine mRNA was similarly affected. Decreased fibrosis may be caused by decreased IL-4 levels "directly" or perhaps is otherwise related to the decreased Th2 response. Vaccination of mice with *S. mansoni* eggs together with IL-12 shifted the cytokine response from a predominantly Th2 to a more Th1-like pattern and also reduced hepatic fibrosis dramatically while having a lesser effect on the granulomatous response.

The reaction to schistosome eggs has long been considered to be stage specific, i.e., the reaction to schistosome eggs was found not to be affected by previous exposure to antigens of adult worms or larvae. We have found that infection with adult male or female worms separately sensitizes fully for granuloma formation around schistosome eggs.

This project will be terminated as of July 1, 1995, with the retirement of the Principal Investigator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00350-13 LPD

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	M.M. Mowatt	Staff Fellow	LPD, NIAID
	J. Yee	Visiting Fellow	LPD, NIAID
	C.G. Clark	Staff Fellow	LPD, NIAID
	L.S. Diamond	Guest Researcher	LPD, NIAID

COOPERATING UNITS (if any)

A. Roger, Dalhousie University, Canada

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Gastrointestinal Parasite Sections

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

3.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For the first time a transient transfection system was developed for *Giardia lamblia* using flanking regions of the glutamate dehydrogenase gene and the luciferase reporter gene. This will enable study of transcription of genes such as those that are induced during encystment, genes which undergo antigenic variation and constitutively expressed genes. Cwp2, a second protein constituent of the *Giardia lamblia* cyst wall was identified and sequenced. Monoclonal antibodies were made to encystment vesicles and cyst walls and were used to identify proteins important in vesicular transport and constituents of the cyst wall.

Entamoeba histolytica contains genes encoding proteins normally associated with the mitochondrion, an organelle this organism appears to lack. Sequence and phylogenetic analysis of two such *Entamoeba* genes has been performed and confirms the specific relatedness. This has major implications for our understanding of the unique biochemistry of this parasite. The same genes have been sought in other parasites thought to lack mitochondria. Analysis of a homologous gene isolated from *Trichomonas* indicates a similar evolutionary history and implies descent from a mitochondrion bearing ancestor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00439-11 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies of Human Filariasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	T. B. Nutman	Section Head	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	S. Mahanty	Senior Staff Fellow	LPD, NIAID
	C. Steel	Biologist	LPD, NIAID
	S. Mawhorter	Medical Staff Fellow	LPD, NIAID
	J.S. McCarthy	Visiting Associate	LPD, NIAID
	P. Zimmerman	NRC Fellow	LPD, NIAID
	T. Moore	Clinical Associate	LPD, NIAID

COOPERATING UNITS (if any)

Indian Council of Medical Research, Madras, India (V. Kumaraswami, R. Prabhakar); MGR Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office, Washington; Centro de Pesquisas Aggeu Magalhães, Dept. of Medicine, University of Iowa, Iowa City, IA (A. Klien); (Cont.)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Helminth Immunology Section

INSTITUTE AND LOCATION

NIAID, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.7

PROFESSIONAL:

1.6

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(Cooperating Units Cont.)

Hospital Vozandes, Quito, Ecuador (R. Guderian); Smith College, Northampton, MA (S.A. Williams); Department of Medicine, Washington University, St. Louis, MO (G. Weil); Division of Parasitic Diseases, CDC, Atlanta, GA (M. L. Eberhard)

Recent clinical studies have defined the presence of individuals with natural immunity to infection with filarial parasites. Some infected individuals may remain microfilaremic over long periods of time, without developing overt lymphatic pathology, but others develop such pathology that can develop either in the setting of persistent microfilaremia or after microfilaremia has cleared.

PCR-based amplifications of parasite DNA in skin snips from patients with onchocerciasis has increased diagnostic sensitivity and specificity, and the assays have been configured into an ELISA format that is field applicable. Similar efforts are underway to detect parasite DNA in lymphatic filariasis, but sensitivity is not yet superior to diagnosis by parasitologic or antigen-detection means.

A treatment trial comparing two or seven 1-week courses of DEC for lymphatic filariasis given over 15 months showed equivalent therapeutic effects but, most importantly, showed for the first time that circulating filarial antigen is an excellent and readable marker of active bancroftian filarial infection. Other studies showed that single yearly doses of ivermectin or DEC are extremely effective in reducing microfilaremia in bancroftian filariasis, and either drug alone (or together) would be appropriate for control programs. In contrast, even three 3-week courses of DEC could cure only about two thirds of expatriates with loiasis; more effective chemotherapy is needed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00483-10 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Drug Resistance and Antigenic Variation in *P. falciparum* Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Thomas E. Wellems	Section Head	LPD, NIAID
Others:	David Peterson	Senior Staff Fellow	LMR, NIAID
	Xin-zhuan Su	Senior Staff Fellow	LMR, NIAID
	Yimin Wu	Research Fellow	LMR, NIAID
	Françoise Guinet	Research Fellow	LMR, NIAID
	Laura Kirkman	Technician	LMR, NIAID

COOPERATING UNITS (if any)

University of Maryland (C. Plowe); ENMRP, Bamako (A. Djimde, O. Doumbo, D. Diallo); Institut Pasteur, Paris (A. Scherf); Hahnemann University, Philadelphia (A. Vaidya)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Genetics and Pharmacology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

6

PROFESSIONAL:

6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations are directed at understanding (1) the mechanism of chloroquine resistance in *Plasmodium falciparum*, (2) the expression and variation of a gene family (*var*) that modulates the antigenic and adhesive character of parasitized red blood cells, (3) certain genes that determine invasion pathways by which malaria parasites invade red blood cells, and (4) a defect in parasite gametogenesis that has been mapped to chromosome 12. A determinant of chloroquine resistance has been localized to a <50 kb segment of the parasite's 7th chromosome. Transcribed genes and DNA sequence data from this segment are being obtained to identify the determinant and the mechanism of resistance. Factors responsible for the spread of drug resistance are being examined in epidemiological studies in Mali. Antigenic variation and cytoadherence of parasitized red blood cells is mediated by a large diverse, family of genes (*var*) that have a copy number of 50-150 per parasite. The *var* family in turn belongs to a superfamily that also includes genes of red cell invasion (*eba-175*, *ebf-1*, DABP genes). Expression and trafficking of the *var* gene products to the erythrocyte surface is under investigation. An unusual pool of sterile RNA transcripts (*gfh*) may be involved in *var* gene rearrangements or expression. A defect in the development of *P. falciparum* male gametes has been traced to a spontaneous mutation in a cultivated parasite line. The mutation has been localized to an 800 kb segment of chromosome 12.

Transfection of parasite erythrocytic stages is now established. Chloramphenicol-acetyl transferase and luciferase have been expressed in intraerythrocytic *P. falciparum* parasites, and homologous integration of pyrimethamine-resistance genes into targeted regions of parasite chromosomes has been achieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00487-09 LPD

PERIOD COVERED

October 1, 1994 - July 1, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Quantitative Parasitology of Schistosome Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.W. Cheever	Chief	Host-Parasite Relations Section
Others:	R. Poindexter	Bio. Lab. Tech.	LPD, NIAID
	F. Lewis		Biomedical Research Institute
	J. Mosimann		ORI

COOPERATING UNITS (if any)

Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade); Biomedical Research Institute, Rockville, MD (Fred Lewis)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Morbidity in schistosomiasis is caused by the host response to schistosome eggs which are deposited in the venous system and carried to the tissues. Pathology is proportional to the number of eggs laid, and it is thus important to understand factors underlying worm fecundity and the extent to which fecundity is reflected by eggs passed in the feces, the measurable indicator of infection intensity in humans.

More detailed examination of the reported decrease in the fecundity of *S. mansoni* in immunodeficient scid mice shows that worms are delayed in maturation, but that when mature, they lay normal numbers of eggs.

This project will be terminated as of July 1, 1995, upon retirement of the Principal Investigator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00494-09 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of T Cell Responses in Human Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Others:	R. Kenney	Senior Research Investigator	LPD, NIAID
	L. Carrera	Guest Researcher	LPD, NIAID
	F. Neva	Chief	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The species of *Leishmania* responsible for past and current epidemics of visceral leishmaniasis (VL) in India is widely assumed to be *Leishmania donovani*. *L. tropica*, a rarely studied Old World species commonly associated with anthroponotic cutaneous disease, was discovered to be responsible for much of the visceral leishmaniasis that is currently epidemic in northeast India. Four of 16 visceral isolates were typed as *L. tropica* by monoclonal antibodies, isoenzymes, and kDNA analysis. *L. tropica* has now been shown to cause at least three distinct disease outcomes: cutaneous lesions, visceral infection associated with unexplained systemic illness recently described in veterans of operation desert storm, and classic kala-azar. The host-, parasite-, and vector-related factors which might explain these vastly different outcomes are currently being explored. Experimental studies on *L. tropica* sp. have been difficult because of the inability to obtain infective forms of the parasite and the absence of animal models. Metacyclic promastigotes of *L. tropica* were identified based on prior observations that the differentiation of promastigotes to an infective form is accompanied by structural modifications of the surface lipophosphoglycan. An anti-IPG monoclonal antibody specific for *L. tropica* was found to identify metacyclic promastigotes. Virulence comparisons of the Indian visceral *L. tropica* were identified based on prior observations that the differentiation of promastigotes to an infective form is accompanied by structural modifications of the surface lipophosphoglycan. An anti-LPG amonoclonal antibody specific for *L. tropica* was found to identify metacyclic promastigotes. Virulence comparisons of the Indian visceral *L. tropica* isolates with isolates from cutaneous patients revealed a difference in their virulence for BALB/c mice, suggesting that their different clinical associations may be at least in part parasite determined.

The ability of *Leishmania* promastigotes to infect macrophages without activating them to produce immunoregulatory cytokines, in particular, IL-12, has recently been suggested as a strategy to delay the development of cell-mediated immunity. *L. major* metacyclic promastigotes failed to stimulate strong induction of any of the monokines examined during in vitro infection of mouse bone marrow-derived macrophages (BMMo). Coexposure of the cells to the parasite and other microbilla stimuli resulted in complete inhibition of IL-12 (p40) mRNA induction and IL-12 release. In contrast, mRNA and protein levels for IL-1 α , IL-1 β , TNF- α , and inducible NO synthase (iNOS) were only partially reduced, and signals for IL-10 and MCP-1/JE were actually enhanced. Selective and complete inhibition of IL-12 was observed in infected BMMo from resistant and susceptible as well as IL-10 knockout mice. Impairment of early IL-12 induction is suggested to underlie the relatively prolonged survival of the parasite that is associated with all leishmanial infections, including those producing selflimiting disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00512-08 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Definition of Filarial and Related Nonfilarial Genes and Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.B. Nutman	Senior Investigator	LPD, NIAID
Others:	J.S. McCarthy	Visiting Associate	LPD, NIAID
	L.H. Elson	Visiting Fellow	FIC LPD, NIAID
	O. Garraud	Visiting Fellow	FIC LPD, NIAID
	P.A. Zimmerman	NRC Fellow	LPD, NIAID
	T. Moore	Clinical Associate	LPD, NIAID
	F. A. Neva	Sr. Investigator	LPD, NIAID
	S. Ramachandran	Visiting Fellow	FIC LPD, NIAID

COOPERATING UNITS (if any)

New England Biolabs, Beverly, Mass (F. Perler, L. McReynolds); Swiss Tropical Institute (E. Lobos); Johns Hopkins University, Baltimore, MD (Alan Scott, Nithya Raghavan); University of Manchester (J. Bradley); New York Blood Center (S. Lustigman); Hospital Vozandes, Quito, Ecuador (Ronald Guderian)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Helminth Immunology Section

INSTITUTE AND LOCATION

NIAID, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.8

OTHER:

.7

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to define and generate filarial and strongyloides proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related helminth species. Recombinant antigens and probes have been identified that: a) encode immunoreactive and potentially protective molecules of *W. bancrofti*; b) can distinguish among related filarial species; c) identify repeated segments of the *W. bancrofti*, *O. volvulus* and *Loa loa* genome; d) are of potential diagnostic importance; and e) are responsible for the induction of immediate hypersensitivity type responses in filarial infections. Further, cDNA libraries of filariform and rhabdiform larvae of *S. stercoralis* have been made and characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00579-06 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Immune Regulation in Toxoplasmosis and other Opportunistic Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Senior Investigator	LPD, NIAID
Others:	E. Denkers	Staff Fellow	LPD, NIAID
	R. Gazzinelli	Senior Staff Fellow	LPD, NIAID
	M. Doherty	IRTA Fellow	LPD, NIAID
	T. Kersten	IRTA Fellow	LPD, NIAID
	E. Grunwald	Howard Hughes Scholar	LPD, NIAID
	S. Bala	Guest Researcher	LPD, NIAID

COOPERATING UNITS (if any)

NIH Clinical Center (J. Kovacs), Uniformed Services University, Bethesda, MD (S. Vogel); Wistar Institute, Philadelphia, PA (G. Trinchieri, M. Wysocka)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

3.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The overall aim of this project is to analyze the immune response to *Toxoplasma gondii* and other opportunistic infections in order to define which cellular immune components and parasite target antigens are involved in the control of infection and its breakdown in immunocompromised hosts.

Progress was made this year in the following areas:

1. Induction of anergy in superantigen reactive T cells during murine infection. A previously characterized population of Vβ5+ T cells expanded by a *T. gondii* superantigen activity was shown to be specifically anergized during the transition from acute to chronic infection.
2. Biochemical characterization and PKC dependence of *T. gondii* monokine-inducing activities. The *T. gondii* molecules responsible for IL-12, IL-10, IL-13 and TNF-γ induction were shown to be heat-stable glyconjugates which differ in their protease sensitivity and dependence on host protein-kinase C.
3. Increased susceptibility of IFN-γ and IL-10 knockout mice to *T. gondii* infections. Mice defective in IFN-γ synthesis were shown to rapidly succumb to infection because of a failure to control parasite growth while IL-10 knockout mice also rapidly died because of the overproduction of proinflammatory cytokines rather than increased parasite levels.
4. Defective parasite-induced IFN-γ and IL-12 synthesis in HIV+ *T. gondii*-infected patients. When stimulated with tachyzoite extracts, PBMC from HIV+ individuals seropositive for *T. gondii* were shown to mount deficient IFN-γ and IL-12 responses while producing normal levels of other parasite-induced monokines.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00629-04 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT

Malaria Studies in Africa

PRINCIPAL INVESTIGATOR

R. W. Gwadz

Assistant Chief

LPD, NIAID

Others:

R. K. Sakai

Senior Scientist

LPD, NIAID

D. C. Kaslow

Section Head

LPD, NIAID

K. D. Vernick

Senior Staff Fellow

LPD, NIAID

G. C. Lanzaro

Senior Staff Fellow

LPD, NIAID

T. E. Wellem

Section Head

LPD, NIAID

COOPERATING UNITS (if any)

National School of Medicine, Bamako, Mali (Dr. Y. Toure); Inst. of Parasitology, Univ. of Rome (Dr. V. Petrarca); Univ. of Maryland School of Medicine (Dr. C. Plowe); European Mol. Biology Lab., Heidelberg, Germany (Drs. F. Kafatos and B. Zheng); Tulane Univ. Dept. Tropical Med. (Drs. D. Krogstad and J. Beier)

LAB/BRANCH

Laboratory of Malaria Research

SECTION

Medical Entomology Section, Unit on International Research

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:

3.3

PROFESSIONAL:

2.7

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

In cooperation with scientists at the National School of Medicine and Pharmacy in Bamako, Mali, the NIH has developed a Malaria Research and Training Center. The goals of the MRTC are two fold. The primary goal is to develop a center of research excellence in Africa where the work is planned, directed and executed by African scientists. A second goal is to provide a facility in a malaria endemic area where the results of laboratory studies at the NIH can be readily applied to studies of malaria in the field. Three main projects are currently under way: 1) As a prelude to the evaluation of strategies for malaria control based on the concept of replacement of vector populations with mosquitoes unable to transmit the malaria parasite, we are examining, in depth, the genetic and biologic structure of vector populations in a series of distinct ecological zones in Mali (West Africa). To facilitate these studies, we are using the tools of molecular genetics directed at microsatellite polymorphisms, restriction length polymorphisms (RFLPs), and ribosomal and mitochondrial DNA. 2) We are applying molecular probes developed at the NIH to the study of the genesis and spread of antimalaria drug resistance in Mali. 3) Site preparation preliminary to the testing of NIH-developed transmission-blocking vaccines are under way in Mali. Special emphasis is being placed on the relative infectivity of human gametocyte carrier population to mosquitoes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00727-01 LPD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recombinant Protein Expression Unit (RPEU)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Kaslow	Section Head	LPD, NIAID
Others:	M. Gozar	Visiting Fellow	LPD, NIAID
	D. Miller	IRTA Fellow	LPD, NIAID
	B. Wood	Pre-IRTA Fellow	LPD, NIAID
	P. Moorthy	Pre-IRTA Fellow	LPD, NIAID

COOPERATING UNITS (if any)

J. Shiloach, Unit Chief, LCBP, NIDDK; J. Coligan, Chief, LMS, NIAID; DMID, NIAID; Malaria Vaccine Development Program, USAID; Immunex Corporation, Seattle, WA (V. Price); MicroGeneSys, Meriden, CN (G. Smith); Catholic University, Nijmegen, The Netherlands (R. Konings and R. Sauerwein); U.S. Army

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Vaccine Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

0.8

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(Cooperating Units Cont.)

(Walter Reed Army Institute of Research, Washington, D.C. -R. Ballou and J. Sadoff); University of Edinburgh, Edinburgh, Scotland (E. Riley); University of Hawaii, Honolulu, HI (G. Hui and S. Chang); Queensland Institute of Medical Research, Brisbane, Australia (A. Saul)

This new initiative is to develop and support a recombinant protein expression unit, RPEU, designed to identify suitable expression systems to produce milligram to gram amounts of adequately folded and post-translationally modified proteins for early stages of vaccine development and for structural studies. The RPEU will consist of 1) a molecular biology group for designing and engineering recombinant DNA for transfection into bacteria, yeast and baculovirus; 2) a production group which will analyze protein production and optimize fermentation conditions from the resultant recombinant bacteria, yeast and baculovirus-infected insect cells; 3) a protein purification and analysis group that will work on post-production process development and will do the preliminary analysis of the resultant recombinant protein for structural and immunological integrity; and 4) a collaborative group that will include LPD and extramural investigators whose major goal will be studying the functional/immunological and structural characteristics of the recombinant malaria parasite proteins produced.

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Rocky Mountain Laboratories
Hamilton, Montana
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00074-23	Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia - Chesebro	18-5
00085-18	Pathogenesis of Aleutian Disease Virus Infection - Bloom	18-6
00086-18	Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses - Portis	18-7
00262-14	Role of Pentraxins in Hamster Physiology - Coe	18-8
00263-14	Structure and Function of the ADV Genome - Bloom	18-9
00265-14	Immunobiology of Scrapie Virus Infection - Race	18-10
00266-14	Genetic Structure of Murine Retroviruses - Evans	18-11
00418-12	Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for Aids - Maury - terminated	18-12
00468-10	Biology of Human AIDS Retrovirus - Chesebro	18-13
00580-06	Biochemistry of Scrapie Pathogenesis - Caughey	18-14
00611-05	Targeting of Hematopoietic Stem Cells for Gene Therapy with Retroviral Vectors - Tumas	18-15
00673-03	Estrogen Hepatotoxicity and Hepatocarcinogenicity in Hamsters - (inactive this year) Coe	18-16

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1995 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1994 to September 30, 1995

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

- Dr. Torben Storgaard, from Department of Pathobiology, Royal Danish Veterinary University, Copenhagen, Denmark, Visiting Fellow working with Dr Marshall Bloom.
- Dr. Martin Oleksiewicz, from Valby, Denmark, Special Volunteer working with Dr. Marshall Bloom.
- Dr. Ryuichi Fujisawa, from Sendai, Japan, Visiting Fellow working with Dr. John Portis.
- Dr. Lionel Wightman, from Department of Microbiology, University of Reading, Berkshire, United Kingdom, working as a Guest Researcher with Dr. Byron Caughey.
- Mr. James Harper, from Massachusetts Institute of Technology, Special Volunteer, working with Dr. Byron Caughey.
- Dr. Winslow Caughey, retired from Colorado Stat University, Special Volunteer, working with Dr. Byron Caughey.

Departures:

- Dr. Lionel Wightman, Guest Researcher, returning to Department of Microbiology, University of Reading, United Kingdom.
- Dr. Gil Katzenstein, IRTA Fellow, expiration of appointment, seeking other employment.
- Dr. Wendy Maury, Staff Fellow, going to Department of Microbiology, University of South Dakota, School of Medicine, Vermillion, SD.

Summer students were Antonette Diaz (Summer IRTA) of Carroll College, Helena, Montana; Shannon Waliser (Summer IRTA) of Carroll College, Helena, Montana; Dirk Williams (Summer IRTA) of Carroll College, Helena, Montana.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1994 to September 30, 1995

OVERVIEW OF RESEARCH IN LPVD

LPVD research projects are aimed primarily at study of viral pathogenic mechanisms and host antiviral defense mechanisms. The main focus is on infection of the central nervous system and the lymphoreticular/hematopoietic systems. Agents under study include: HIV, murine retroviruses, rabies virus, Aleutian mink disease virus and scrapie.

Foreign and chimeric PrP genes have been expressed *in vitro* in mouse neuroblastoma cells with and without scrapie infection. In uninfected cells expressing hamster PrP, a unique dimer PrP was identified with properties analogous to both protease-sensitive and protease-resistant PrP. This new form might provide clues to the mechanisms of conversion between these two forms which occurs during scrapie pathogenesis.

Protease-resistant forms of prion protein (PrP-res) have been generated in a cell-free *in vitro* system by mixing radiolabelled protease-sensitive PrP with unlabeled PrP-res from scrapie brain tissue. There appears to be some species specificity to this reaction and also some differences related to the use of different scrapie strains from the same animal species. This *in vitro* conversion reaction appears to be similar to nucleation dependent polymerizing processes seen in other nontransmissible amyloid diseases, and may be an important aspect of *in vivo* pathogenesis. The roll of this conversion in transmission of scrapie infection is currently being studied.

Two murine leukemia viruses which cause degenerative brain disease have been analyzed and compared. These viruses use different receptors, but in both cases the envelope gene is a primary determinant of CNS tropism and disease induction (neurovirulence). However, sequences causing neurovirulence are distinct from those causing neurotropism as some viruses which infect these same brain regions do not cause clinical disease. Current efforts are aimed at developing *in vitro* culture systems which will allow study of the pathogenic mechanisms involved.

The magnitude of HIV replication in human macrophages was found to be determined by the V1 and V2 hypervariable regions of the envelope gene. V1 and V2 sequences of high level replicating strains allowed virus to spread throughout macrophage cultures, whereas sequences of low level replicating strains failed to allow spread to new cells after initial infection. These two viral phenotypes may have unique pathogenic roles in AIDS patients.

The Rfv-3' gene is one of several host genes required for recovery from disease in Friend virus-infected mice. This gene facilitates the host humoral antibody response to FV infection, and results in lowering of levels of viremia and virus expression in spleen cells at 3-4 weeks postinfection. Recent studies using microsatellite mapping methods have identified the location of the Rfv-3 gene on mouse chromosome 15 near to the Ly6 T cell antigen locus as well as several cytokine receptor genes. More precise mapping may facilitate characterization of the mechanism of action of this gene.

A novel mouse retroviral vector (pSFF) was used to deliver and express a foreign gene (mutated PrP) in murine hematopoietic stem cells. Expression was detected in blood in several hematopoietic lineages and in stem cells purified from bone marrow for up to 7 months following bone marrow transplantation.

Endogenous murine retroviral sequences participating in recombination with exogenously acquired retroviruses often generate viruses with an altered host range. Antigenic markers and corresponding

mutations at the sequence level have identified distinct endogenous viral genes involved in these events. Recent studies indicate that sequences in the nucleocapsid gene of the exogenous virus may affect magnitude of infection in particular cell types *in vivo* and also influence which endogenous loci are used in recombination events.

Aleutian mink disease virus (ADV) from a natural outbreak in Utah has been characterized by PCR and DNA sequencing of the hypervariable region of the capsid protein gene. The new isolate was > 97% identical to type 1 nonpathogenic viruses. This is the first high virulence virus in this category. Viral determinants of virulence and host range appear to be extremely complex.

Rabies virus has been shown to infect murine macrophage cell lines, primary macrophages and microglia. Human mixed glial cell cultures and astrocyte cultures were also infected. Persistent viral infection of mouse macrophages resulted in production of virus with decreased virulence for CNS disease. Current efforts are aimed at identifying sites of persistent infection *in vivo*.

In Syrian hamsters sex hormones control serum levels of female protein (FP) which is involved in nonspecific inflammatory responses and amyloid formation in liver, kidney, heart and other tissues with aging. In comparison, Turkish hamsters have FP which is hormonally regulated but has serum levels 1/10 that found in Syrian hamsters. Amyloidosis was not found in Turkish hamsters even after hormonal treatment. The results suggest that the magnitude of serum FP levels is a critical factor in amyloidogenesis associated with aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00072-24 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: D. L. Lodmell

Scientist Director

LPVD, NIAID

Other:

Nancy B. Ray, Ph.D.

IRTA Fellow

LPVD, NIAID

COOPERATING UNITS (if any)

Jean Smith, M.S.

CDCP

Joseph J. Esposito

CDCP

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal objectives of this research project are six-fold: 1) to determine host and viral factors which influence the genetically controlled resistance of inbred mouse strains to street rabies virus (SRV) 2) to determine if an *in vivo* laboratory model for persistent rabies virus infection can be established with subsequent goals of determining in which cell(s) the virus (genome) is sequestered and whether the virus can be activated to produce clinical disease 3) to understand the role of macrophages in rabies virus infections 4) to compare the protective capabilities of recombinant vaccines expressing different or multiple structural proteins of the rabies virus against genetic variants of rabies virus that have been isolated worldwide 5) to understand the complex interaction between microglial cells, lymphocytes and cytokines in the pathogenesis of rabies virus infections and 6) to determine the feasibility of using "naked DNA" vaccines to protect mice against a global spectrum of rabies virus variants. We have determined that both tissue culture adapted and non-tissue culture adapted wild strains of rabies viruses replicate in primary murine bone marrow-derived macrophages and murine and human macrophage-like cell lines. Similar viruses also have been shown to replicate in primary murine microglial cells, primary feline and human mixed glial cells, and in primary human astrocytes. Rabies viruses harvested from persistently infected cells were shown to have specifically adapted to each cell type. Furthermore, after extended passage, viruses released from persistently infected macrophages lost virulence as determined by their inability to kill intracranially inoculated mice. Recombinant vaccinia viruses expressing either the glycoprotein (G), or both the G and nucleoprotein (N) (GN) of the challenge virus strain (CVS) of rabies virus protected mice ($\geq 95\%$) against a global spectrum of rabies virus variants. The concurrent expression of G and N in a vaccine was no more protective than the expression of G alone. A recombinant virus expressing only N was less protective. Antibody prepared against the G of the strains used in the vaccines neutralized all variant viruses, and sera from mice infected with any one variant cross-neutralized all of the other viruses. Attempts to isolate infectious virus (genome) from persistently infected mice are ongoing. Preliminary data indicate that RT-PCR is more sensitive than *in vitro* amplification of infectious virus, particularly in mice which have high titers of serum and brain neutralizing antibody. Furthermore, sets of N and G primers have been prepared which detect all viruses that were used to establish persistent infections. Initial studies have determined that neutralizing antibody is elicited following immunization with "naked DNA" vaccines encoding the CVS G gene. Ongoing studies will establish optimal conditions (route of vaccination, concentration of DNA, eukaryotic expression vector) for protection of mice against a global spectrum of rabies virus variants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00074-23 LPVD						
PERIOD COVERED October 1, 1994 to September 30, 1995								
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Genetically Controlled Mechanisms of Pathogenesis and Recovery in Friend Retrovirus-Induced Leukemia								
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: B. Chesebro</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LPVD, NIAID</td> </tr> <tr> <td>Other: K. Hasenkrug</td> <td>Staff Fellow</td> <td>LPVD, NIAID</td> </tr> </table>			PI: B. Chesebro	Chief	LPVD, NIAID	Other: K. Hasenkrug	Staff Fellow	LPVD, NIAID
PI: B. Chesebro	Chief	LPVD, NIAID						
Other: K. Hasenkrug	Staff Fellow	LPVD, NIAID						
COOPERATING UNITS <i>(if any)</i> Dr. Wayne Frankel, Jackson Labs, Bar Harbor, Maine.								
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840								
SECTION								
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892								
TOTAL MAN-YEARS: 2.9	PROFESSIONAL: 1.3	OTHER: 1.6						
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> <input checked="" type="checkbox"/> (c) Neither This is a non-clinical AIDS-related project. </td> </tr> </table>			<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither This is a non-clinical AIDS-related project.			
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither This is a non-clinical AIDS-related project.						
SUMMARY OF WORK <i>(Use standard unredacted type. Do not exceed the space provided.)</i> <p> Friend virus (FV) is a complex of 2 mouse retroviruses which induce rapid erythroleukemia in many strains of adult mice. Various genes in susceptible mice can modify the outcome of FV infection. Our laboratory has identified 5 such genes including 4 major histocompatibility complex (MHC) regions (D, IA, IE and T) and one non-MHC gene, Rfv-3. These genes all influence the host immune response to FV and FV leukemia cells and can even induce spontaneous recovery from leukemia or facilitate successful protective vaccination against challenge with live FV or FV leukemia cells. </p> <p> This year, using microsatellite mapping methods, the location of the Rfv-3 gene was identified to be on mouse chromosome 15. Rfv-3 is known to influence the ability of mice to make a humoral antibody response to FV during the course of active infection. Mice with the Rfv-3⁻ allele make anti-FV antibodies and clear FV viremia 2-3 weeks after infection. This is necessary, but not sufficient, for recovery from FV leukemia. The mechanism of the Rfv-3 gene function on the host immune response is not known, but the location of the gene indicates that it is near a T cell antigen gene (Ly6) and 3 cytokine receptor genes (IL2rb, IL3rb1, and IL3rb2). Future experiments will be aimed at identifying the mechanism of action of this gene. </p> <p> Other experiments have shown that mice lacking the Rfv-3⁻ allele can still recover from FV leukemia if they are inoculated with high doses of anti-FV antibody. This antibody therapy was effective even if initiated after the peak of virus spread <i>in vivo</i>. However, recovery associated with antibody therapy also required T cell-mediated immune mechanisms because no recovery was observed in CD4⁻ or CD8-depleted mice. These results indicate that recovery from Friend retroviral infection is dependent on the cooperative effects of several difference immune mechanisms including T helper cells, cytotoxic T and antibodies, and antibodies. The results imply that similar mechanisms might be effective in treatment of HIV infection with antibody therapy. </p>								

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00085-18 LPVD												
PERIOD COVERED October 1, 1994 to September 30, 1995														
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Pathogenesis of Aleutian Disease Virus Infection														
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. E. Bloom</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 33%;">LPVD, NIAID</td> </tr> <tr> <td>Other: Linda Dworak</td> <td>Staff Fellow</td> <td>LPVD, NIAID</td> </tr> <tr> <td>Torben Storgaard</td> <td>Visiting Fellow</td> <td>LPVD, NIAID</td> </tr> <tr> <td>Martin Oleksiewicz</td> <td>Special Volunteer</td> <td>LPVD, NIAID</td> </tr> </table>			PI: M. E. Bloom	Medical Officer	LPVD, NIAID	Other: Linda Dworak	Staff Fellow	LPVD, NIAID	Torben Storgaard	Visiting Fellow	LPVD, NIAID	Martin Oleksiewicz	Special Volunteer	LPVD, NIAID
PI: M. E. Bloom	Medical Officer	LPVD, NIAID												
Other: Linda Dworak	Staff Fellow	LPVD, NIAID												
Torben Storgaard	Visiting Fellow	LPVD, NIAID												
Martin Oleksiewicz	Special Volunteer	LPVD, NIAID												
COOPERATING UNITS <i>(if any)</i> State Veterinary Institute for Virus Research, Lindholm, Denmark Royal Veterinary and Agricultural University, Copenhagen, Denmark														
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840														
SECTION														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892														
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.6	OTHER: 0.9												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td colspan="2">This is a non-clinical IIDEA project.</td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews	This is a non-clinical IIDEA project.				
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews	This is a non-clinical IIDEA project.													
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>Persistent infection of mink with Aleutian mink disease parvovirus (ADV) causes disturbances of immune regulation, including polyclonal hypergammaglobulinemia, plasmacytosis, immune complex disease, interstitial and glomerulonephritis and exceedingly high levels of anti-ADV antibodies. The spectrum of findings resembles those associated with a Th2 pattern of cytokine responses and previous work implicates a possible role for IL-6. The scope of this project is to elucidate mechanisms by which ADV infection results in this unusual disorder.</p> <p>The U937 cells used previously in antibody-dependent enhancement (ADE) of ADV infection were found to be mixture of 2 monocytic cell lines, >90% K562 and <10% U937. Experiments with pure cell populations indicated that K562, but not U937 cells were susceptible to ADV via ADE. Infection was via the Fc gamma RII receptor. IL-6 was elaborated by K562 cells in response to ADV infection.</p> <p>In order to evaluate the role of selected cytokines in the immune disorder associated with ADV infections, RT-PCR was done on mRNA from tissues from infected mink. Degenerate primers for IL-4, IL-5, IL-10, IL-12 p35 and IL-12 p40 were based on consensus sequences from human, mouse, cat, rat, cow and pig. Appropriate sized PCR products were obtained and have been cloned. DNA sequencing is currently being done.</p> <p>In order to identify early target cells for viral replication, mink were infected both intranasally (IN) and intraperitoneally (IP) with ADV. Progressive disease develops by either route, but progression seems slower after IN infection. Earliest evidence of ADV DNA was localized by PCR to draining lymphoid tissues.</p> <p>In order to compare the subcellular localization of ADV proteins and nucleic acids in permissive and restricted infection, specific antisera for nonstructural (NS) and capsid proteins have been prepared and conjugated to fluorochromes. Asymmetric PCR has been used to develop fluoresceinated strand specific probes for fluorescence in situ hybridization (FISH). Preliminary work on synchronized cells with multi-channel epifluorescence and confocal microscopy suggests that NS1 and NS2 co-localize, but that NS and capsid proteins are spatially segregated within the nucleus of permissively infected cells.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00086-18 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. L. Portis Medical Officer LPVD, NIAID

Other: Richard Bessen IRTA Fellow LPVD, NIAID
Ryuichi Fujisawa Visiting Fellow LPVD, NIAID
Shelly Robertson Pre-IRTA Fellow LPVD, NIAID

COOPERATING UNITS (if any)

Laboratory of Molecular Structure, NIAID

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.2

PROFESSIONAL:

3

OTHER:

2.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Retroviruses, including HIV and HTLV, cause neurological disease. The goal of this project is to understand how retroviruses adversely affect the central nervous system. We are utilizing mouse retroviruses which cause rapid neurologic diseases (ie. 2-3 weeks after inoculation) and have used this system to study the interaction between virus and brain.

Entry of virus into the brain: A non-structural glycosylated form of the viral *gag* polyprotein (glycogag) has long been recognized to be highly conserved among murine, feline and some primate retroviruses, but its function is as yet unknown. We have found that expression of this protein is a critical determinant neuroinvasiveness, and that this protein functions to enhance the spread of virus *in vivo*. Nonpathogenic mutant viruses which lack this protein rapidly generate revertants which have regained their pathogenicity for the brain. Sequence analysis of the revertants reveals a consistent mutation which generates a new start site for translation producing a slightly truncated protein. This indicates strong selective pressure favoring viruses which express glycogag. We are interested in understanding its function at the molecular level.

In vitro model of neurodegeneration: We are continuing our efforts to develop a system whereby one can study at the molecular level the mechanism of retrovirus-induced neurodegeneration. We have initiated a study of embryonic cortical neurons cultured on a glial bed. This system has been used successfully by others to study neurotoxicity induced by excitatory neurotransmitters as well as hypoxia. These neurons are relevant since they are also susceptible to degeneration in virus-infected mice. Preliminary results suggest that supernatants from retrovirus-infected but not from uninfected pure microglial cultures cause neurotoxicity in this system. We plan to extend these studies to the analysis of microglia expressing individual viral genes and have constructed the appropriate expression vectors.

Envelope gene: Two chimeric murine retroviruses which utilize the same receptor but differ in their envelope sequences have both been shown to infect the brain, and appear also to infect the same cell types in the brain. Both induce an intense diffuse astrocytosis, but only one causes clinical neurologic signs. We are very interested in this paradigm because of its analogy to brain infection by HIV, in which HIV envelope sequences of macrophage tropic viruses have been recovered from both demented and non-demented AIDS patients. We are currently looking for morphologic correlates of neurovirulence in these mice and are currently analyzing cytokine profiles in the brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00262-14 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Pentraxins in Hamster Physiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. E. Coe Medical Officer LPVD, NIAID

Other:

COOPERATING UNITS (if any)

Drs. B. Canquihem & P. Pevet, Strasbourg, France; Dr. B. Dowton, Wash. Univ. Med. School, St. Louis, MO; Dr. K. Ishak, AFIP, Washington, D.C.; Dr. U. Nilsson, Uppsala, Sweden; Dr. Mortensen, OSU, Columbus, OH; Dr. D. Johnson, U. Kansas Med. Center, Kansas City; Dr. Gary Nelsestuen, U. Minn., St. Paul; Dr. H. Gewurtz, Rush Med. Center, Chicago, IL; Dr. G. Prince, Bethesda, MD; Dr. W. Cieplak, RML; Dr. Wm. Hadlow, Hamilton, MT.

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

NIAID, NIH, Bethesda, MD 20892

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Syrian hamsters have a remarkable serum protein called female protein (FP) that is controlled by sex hormones. FP is preferentially expressed in female hamsters that normally have 2-3 mg FP/ml serum, whereas, normal male serum has 100- 300-fold less. This protein is a homolog of two human serum proteins: C reactive protein (CRP) and serum amyloid P (SAP) component. This family of proteins is called pentraxins because of their unusual structure – a pentamer of 5 non-covalently monomer subunits in a disk-like arrangement. Pentraxins are ancient proteins, have changed little during evolution and are found in most animals, even quite primitive ones as the horse shoe crab. These findings would suggest an important function for these proteins; however, a *raison d'être* has not yet been described. FP shares many properties with CRP and SAP such as Ca^{++} dependent phosphorylcholine binding, complement fixation, acute phase responsiveness and hepatic synthesis. FP is also a constituent of amyloid. Amyloid deposits in humans are most commonly found in brain of patients with Alzheimer's disease. However, in Syrian hamsters amyloidosis is unusual because it is very common and also is a sex-limited disease, preferentially expressed in females. Amyloid deposits in vital organs result in a shorter longevity of the female (versus male) Syrian hamster, an unusual phenomenon in mammals. Amyloidosis in Syrian hamsters can be directly related to serum FP levels in both male and female hamsters. Thus when FP synthesis (i.e., serum levels) is experimentally manipulated by hormonal means, expression of amyloidosis in both sexes is enhanced with high FP levels and is diminished with low levels. The Turkish hamster (*Mesocricetus brandti*) is a close relative of the Syrian hamster (*Mesocricetus auratus*) and also has a serum FP. The Turkish hamster's FP is antigenically identical to Syrian hamster FP and shares identical amino acid sequences. Although Turkish hamster FP is under similar sex-hormone control, the normal female serum levels are only 1/10 that found in Syrian hamster females. Commensurate with this low serum FP, amyloidosis was not found in aging normal Turkish hamsters. Amyloidosis was rare even after estrogen treatment, which markedly accelerates amyloidosis in Syrian hamsters. Turkish hamsters also differed from Syrian hamsters because prolonged estrogen treatment did not result in renal adenocarcinoma. On the other hand, normal old Turkish hamsters were found to have a high incidence of hepatocellular carcinoma (HCC). The appearance of HCC in Turkish hamsters was not enhanced by estrogen treatment as found in Armenian hamsters, and exogenous estrogen did not appear to have any acute hepatotoxic effect similar to that observed in Armenian hamsters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00263-14 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: M. E. Bloom Medical Officer LPVD, NIAID

Other: Torben Storgaard Visiting Fellow LPVD, NIAID
Katrina Oie Special Volunteer LPVD, NIAID

COOPERATING UNITS (if any)

Department of Veterinary Virology, Royal Danish Veterinary University, Copenhagen, Denmark
Utah Fur Breeder's Agricultural Cooperative, Midvale, Utah

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

1.0

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ongoing purpose of this project is to identify genomic differences among various strains of Aleutian mink disease parvovirus (ADV) and to relate these differences to viral host range and pathogenicity.

Viruses from an ongoing outbreak of ADV infection in Utah were studied. PCR was used to isolate and characterize the 161 bp core hypervariable region of the capsid protein gene. Sequence of specimens from multiple mink and feral raccoons involved in the outbreak were very closely related to each other (>97%). Furthermore, they were more closely related to the type 1 nonpathogenic ADV-G (>97%) than to pathogenic strains such as the type 2 ADV-Utah, the type 3 Danish ADV-K or an isolate from feral skunks (all <90%). Expanded sequencing of a virus derived from this outbreak (ADV-TR) indicated that at other residues, the capsid protein gene differed from ADV-G and more closely resembled pathogenic strains of ADV. Thus, a virus with a type 1 hypervariable region can be pathogenic, however, these results indicate that viral determinants for pathogenicity and host range may be extremely complex.

ADV-TR was highly pathogenic for both Aleutian and non-Aleutian genotype mink. Pathogenic strains of ADV, including ADV-TR, induced antiviral antibodies and viremia, but no evidence of pathology in raccoons. ADV-TR could be passaged from infected raccoons to mink. Thus, raccoons may play a role in maintenance and transmission of ADV.

Other work designed to examine function of the transcription unit from the capsid protein P36 promoter has been undertaken. First strand cDNAs have been prepared from cells transfected with several parvovirus promoter constructs for use in 5'-RACE to compare the transcription initiation site under constitutive and trans-activated conditions. The 5'-RACE fragments have been cloned and are being characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00265-14 LPVD												
PERIOD COVERED October 1, 1994 to September 30, 1995														
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Immunobiology of Scrapie Virus Infection														
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R. E. Race</td> <td style="width: 33%;">Research Veterinarian</td> <td style="width: 33%;">LPVD, NIAID</td> </tr> <tr> <td>Other: B. Caughey</td> <td>Research Chemist</td> <td>LPVD, NIAID</td> </tr> <tr> <td>S. Priola</td> <td>Staff Fellow</td> <td>LPVD, NIAID</td> </tr> <tr> <td>B. Chesebro</td> <td>Chief</td> <td>LPVD, NIAID</td> </tr> </table>			PI: R. E. Race	Research Veterinarian	LPVD, NIAID	Other: B. Caughey	Research Chemist	LPVD, NIAID	S. Priola	Staff Fellow	LPVD, NIAID	B. Chesebro	Chief	LPVD, NIAID
PI: R. E. Race	Research Veterinarian	LPVD, NIAID												
Other: B. Caughey	Research Chemist	LPVD, NIAID												
S. Priola	Staff Fellow	LPVD, NIAID												
B. Chesebro	Chief	LPVD, NIAID												
COOPERATING UNITS <i>(if any)</i> Dr. Al Jenny, U.S.D.A., Ames, IA; Dr. Lyle Miller, Iowa State University; Dr. Randall Cutlip, U.S.D.A.; Dr. Michael Oldstone, Scripps Clinic, San Diego CA; Dr. Jean Manson, Edinburgh.														
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840														
SECTION														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892														
TOTAL MAN-YEARS: 3.3	PROFESSIONAL: 2.2	OTHER: 1.1												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>Scrapie is a spongiform encephalopathy of sheep and goats which can be transmitted experimentally to several other animal species. Similar diseases are recognized in cattle and humans. No etiologic agent has been identified. However, the proteinase K resistant form (PrP-res) of an endogenous protein designated prion protein (PrP) purifies with infectivity and is important to disease pathogenesis.</p> <p>We developed a sensitive assay for PrP-res and utilized it to diagnose scrapie in sheep. Analysis based on PrP-res detection was much more accurate and less subjective than the currently used method of diagnosis based on the microscopic evaluation of brain. We also showed that PrP-res analysis of spleen or lymph node was nearly as accurate as analysis of brain. We are determining if PrP-res accumulates prior to clinical disease in lymph node. In addition we have analyzed placenta from sheep to determine if PrP-res accumulates prior to clinical disease in this easily obtained tissue. If PrP-res accumulates prior to clinical disease in either placenta or lymph node an ante mortem test for infection may for the first time be available. We are also using PrP-res analyses to test tissues from cattle in order to determine if spongiform encephalopathy currently exists in U.S. cattle and, thereby, whether an epidemic similar to BSE in Great Britain is possible in the U.S.A. PrP-res analysis should also be relevant for diagnosis of the human disease counterparts.</p> <p>The influence of specific PrP gene sequences on interspecies transmission of spongiform encephalopathies is also being studied. To do so, we have expressed various mouse-hamster PrP constructs in scrapie-infected mouse neuroblastoma (MNB) cells and are now analyzing them in mice and hamsters to determine if species tropism has been altered. Mouse neuroblastoma cells are also being used to study the normal function of the PrP protein as well as to identify factors which might account for the biochemical changes which lead to the conversion of the endogenous PrP protein to the disease associated PrP-res form.</p> <p>Similar experiments are being done in vivo using transgenic mice which express hamster PrP and transgenic mice which do not produce PrP (PrP null mice).</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00266-14 LPVD
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Genetic Structure of Murine Retroviruses		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> PI: L. H. Evans Chemist LPVD, NIAID Other: M. Lavignon Fogarty Fellow LPVD, NIAID		
COOPERATING UNITS <i>(if any)</i> 		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION 		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided..)</i> <p>Genetic variation as a result of point mutation or recombination occurs in all retrovirus infections including avian, murine and human retroviruses such as HIV. Such variation often has a profound influence on the infectious, replicative and pathogenic properties of the viruses. Mice inoculated with certain retroviruses generate variant retroviruses with altered infectious properties. The variants are the result of recombination of the inoculated virus with endogenous retroviral gene sequences of the mouse. The variants utilize a different cell surface receptor for infection, thus altering the infectious host range and have been implicated in a variety of proliferative diseases in the mouse. There exist 30 to 40 distinct retroviral sequences in the mouse genome which encode the receptor binding specificity exhibited by the variants, however which of these participate in recombination is unknown. Identification of the participants in the recombination process is essential to its understanding. It is clear from previous analyses of virion RNAs that the variants are not all derived from the same endogenous sequence. Furthermore, we have identified two different antigenic subgroups of the variants which correspond to two different subgroups of endogenous sequences and have found that different retroviruses preferentially recombine with different sets of endogenous sequences. Our most recent studies have been directed at: 1) the precise identification of the endogenous sequences which participate in recombination and: 2) the identification of viral genes of the inoculated viruses which influence recombination with particular sets of endogenous sequences. Sequence comparisons of the variant viruses with retroviral gene sequences from uninfected mice have identified particular endogenous genes which give rise to the recombinant viruses. Further analyses will reveal additional endogenous genes which participate in recombination and the frequency with which they do so. With regard to the viral gene(s) which influence the specificity of recombination, we have examined variants generated after infection of mice by chimeric viruses constructed between two retroviruses which clearly differ in the subgroups of variants generated. We have found that a region of the genome which encompasses the gene encoding the nucleocapsid protein of the virus strongly influences the recombinant subgroups identified in infected mice. Further studies may more precisely determine a smaller sequence responsible for this effect and may identify other regions of the genome which influence the specificity of recombination.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00418-12 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular biology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. Maury Staff Fellow LPVD, NIAID

Other: B. Chesebro Chief LPVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is focussed on the regulation of expression of the equine infectious anemia virus (EIAV) in monocytes and differentiated macrophages. The results indicate that virus expression is strongly influenced by sequences in the viral LTR which interact with members of the ets-family of transcription factors. Variation in these sequences correlates with variation in viral tropism for cell lines and macrophages *in vitro*.

The project was terminated after the departure of Dr. Maury to the University of South Dakota Medical School.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00468-10 LPVD
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (<i>80 characters or less. Title must fit on one line between the borders.</i>) Biology of Human AIDS Retrovirus		
PRINCIPAL INVESTIGATOR (<i>List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> PI: B. Chesebro Chief LPVD, NIAID Other: K. Toohey IRTA Fellow LPVD, NIAID		
COOPERATING UNITS (<i>if any</i>) Diane Griffin, M.D., Department of Neurology, Johns Hopkins University School of Medicine; Richard Johnson, M.D., Department of Neurology, Johns Hopkins University School of Medicine; Chris Power, M.D., Department of Neurology, University of Manitoba, Winnipeg, Manitoba, Canada.		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.1	PROFESSIONAL: .4	OTHER: 1.7
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div> <div style="text-align: right; margin-top: 5px;"> This is a non-clinical AIDS-related project. </div>		
SUMMARY OF WORK (<i>Use standard unredacted type. Do not exceed the space provided..</i>) Human immunodeficiency virus (HIV) causes a variety of clinical manifestations such as opportunistic infection, Kaposi's sarcoma, wasting syndrome, and neurological defects. The main goal of this project is to determine the role of variation in viral sequences in causing these different syndromes. In the past year brain samples from a prospective clinical study on HIV dementia were used to identify significant sequences in HIV envelope C2 and V3 regions which differed in demented and nondemented AIDS patients. Sequences from both groups were also cloned in infectious plasmids and were found to be macrophage-tropic in all cases. These results are consistent with the interpretation that most HIV strains present in brain are macrophage-tropic strains which infect brain microglial cells, but HIV dementia appears to correlate with the appearance of a unique subset of macrophage-tropic viruses which cause dementia via as yet unknown mechanisms. More recent studies have focussed on <i>in vitro</i> infection of human macrophages by HIV strains which vary in replication levels in macrophages. This variation was found to occur only in macrophages and not in lymphocytes, and it correlated with sequence differences in the envelope V1 and V2 regions. Immunostaining of virus-positive cells at various times after infection showed that high replication levels were due to viral spread in the macrophages whereas low replication levels were seen in viruses which were unable to spread to new cells after initial infection. These two replication phenotypes have been seen in primary AIDS patient HIV isolates and might play different roles in <i>in vivo</i> pathogenesis.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00580-06 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Scrapie Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Caughey, Ph.D.

Research Chemist

LPVD, NIAID

Other: Bruce Chesebro, M.D.

Chief

LPVD, NIAID

Gil Katzenstein, Ph.D.

IRTA Fellow

LPVD, NIAID

Suzette Priola, Ph.D.

IRTA Fellow

LPVD, NIAID

Richard Bessen, Ph.D.

IRTA Fellow

LPVD, NIAID

COOPERATING UNITS (if any)

Peter Lansbury, Department of Chemistry, MIT, Cambridge, MA

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.6

PROFESSIONAL:

2.6

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Scrapie and other transmissible spongiform encephalopathies (TSE) are transmitted by an infectious agent that resembles a virus in that it replicates *in vivo* and has distinct strains. However, the agent has been proposed to contain only PrP-res, a neuropathogenic, protease-resistant form of prion protein derived post-translationally from normal, protease-sensitive PrP (PrP-sen). To simplify the analysis of the conversion mechanism and the relationship of PrP-res formation to TSE agent replication, we developed a defined cell-free system in which PrP-sen is converted to protease-resistant forms similar to scrapie associated PrP-res. This conversion required the presence of preexisting PrP-res, providing the first direct evidence that PrP-res derives from interactions between PrP-sen and PrP-res.

We have now studied the species specificity of this cell-free reaction using mouse, hamster, and chimeric PrP molecules. Species specific conversions were observed which correlated with the relative transmissibilities of these strains of scrapie between mice and hamsters. Conversion experiments performed with chimeric mouse/hamster PrP-sen precursors indicated that localized primary sequence differences between PrP-sen and PrP-res strongly affected the conversion reaction. The species specificity in the conversion of PrP-sen to protease-resistant forms may be the molecular basis for the barriers to interspecies transmission of scrapie and other TSEs *in vivo*.

We also tested whether strain specific properties of PrP-res are transmitted to PrP-sen during formation of new PrP-res. PrP-res isolated from the brains of hamsters with the HY and DY strains converted hamster PrP-sen to two distinct sets of PrP-res species. This provided evidence that the self-propagation of PrP-res polymers with distinct 3-D structures may be a molecular basis for scrapie strains.

The properties of the activity that converts PrP-sen to PrP-res were analyzed. The activity was stimulated by treatments with 2-3 M guanidine HCl. These treatments solubilized a majority of the PrP from otherwise insoluble PrP-res preparations, but the converting activity remained associated with insoluble particles. This provides evidence that the converting activity requires a multimeric form of PrP-res and argues strongly against the commonly espoused heterodimer model for PrP-res formation and scrapie agent replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00611-05 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Targeting of Hematopoietic Stem Cells for Gene Therapy with Retroviral Vectors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.B. Tumas, D.V.M., Ph.D. Staff Fellow LPVD, NIAID

Other: B. Chesebro, M.D. Chief LPVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to find effective vectors for delivery and expression of foreign genes in hematopoietic stem cells and their differentiated progeny cells.

A retroviral vector (pSFF) derived from murine Friend spleen focus forming virus was used to transduce murine hematopoietic stem cells and express a cell surface marker protein, mutated murine prion protein, in vitro and in vivo after transplantation. To enhance retroviral vector integration in bone marrow cells, mice were treated with 5-fluorouracil (5-FU) to increase stem cell mitotic activity which peaked on day 8 post-5-FU. The infectivity titer of the vector, pSFF-mPrP-3F4, was determined by a novel assay in which antigen-positive foci of infected cells were detected after replication and spread of the vector in cultures of mixed packaging cell lines. Infection of Sca-1+/Lineage^{neg-low} cells with pSFF-mPrP-3F4 resulted in marker protein expression in 40% of the progeny cells after 7 days of culture. Transplantation of marrow cells or sorted Sca-1+/Lineage^{neg-low} cells transduced with vector resulted in 3F4-positive mPrP expression in 11-37% of donor-derived peripheral blood leukocytes at two weeks. Though the percentage of 3F4-positive blood cells gradually declined, at 28 weeks 23% of recipient mice still maintained expression of the marker gene. Expression was observed in lymphoid, myeloid and erythroid lineages and was detected in Sca-1+/Lineage^{neg-low} marrow cells. The multi-lineage, high frequency expression observed suggests that pSFF may have utility in gene therapy directed to hematopoietic stem cells and their differentiated progeny.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00673-03 LPVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Estrogen Hepatotoxicity and Hepatocarcinogenicity in Hamsters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. E. Coe Medical Officer LPVD, NIAID

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TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

This project is temporarily inactive.



**LABORATORY OF VIRAL DISEASES
1995 ANNUAL REPORT
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LABORATORY OF VIRAL DISEASES
ANNUAL REPORT, 1995
SUMMARY

Members of the Laboratory of Viral Diseases carry out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. These studies are intended to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of viral infections. Current topics of basic research include viral entry into cells, regulation of gene expression, mechanisms of DNA replication, assembly and transport of viral proteins and particles, action of viral growth factors, determinants of viral virulence, and viral humoral and cellular immunity. Applied areas of investigation include development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of DNA and RNA viruses including human immunodeficiency virus. I have summarized recent research findings, administrative changes, and individual honors below.

REGULATION OF VIRAL GENE EXPRESSION. Regulated gene expression occurs in virtually all biological systems. Historically, viruses have served as models because of their relatively small size and the programmed nature of their replication. Some aspects of viral gene regulation, however, are unique and thus represent potential targets of therapeutics. In addition, the optimal use of viruses as expression vectors requires a thorough understanding of viral mechanisms.

Herpes simplex virus (HSV). HSV is used as a model system for the study of the biochemical mechanisms involved in the regulation of RNA polymerase II-directed transcription. The three major classes of viral genes (IE, E, and L) are transcribed under distinct conditions representing unique requirements for the initiation and regulation of each promoter class. This regulation is dependent upon the interaction of viral and cellular factors that determine the recognition and transcriptional activation of particular genes. Thus the identification, isolation, and characterization of these components can lead to further understanding of the mechanisms by which the virus controls its genetic expression as well as to insights into basic cellular processes.

Analysis of C1 transcription factor. The IE genes of HSV are regulated by the assembly of a multiprotein complex containing several viral and cellular factors (Oct-1, α TIF, and the C1 factor). Using protein microsequence data, cDNA clones encoding the human C1 factor were isolated and characterized. The protein, a novel transcription factor of 220 kD, was shown to be ubiquitously expressed, associated with the nuclear matrix in most cell types, and localized to specific subdomains in the nucleus. The C1 factor was also shown to be highly conserved as the activity was detected in several species. Therefore, partial cDNA and genomic clones were isolated that encode homologs of the human factor. These clones are presently being investigated for the study of the cellular functions of the C1 factor as well as to ascertain the

significance of this protein in the expression of the HSV IE *in vivo*. These comparative genetic systems will allow more flexible and rapid analysis of the C1 factor. As the interactions of the C1 factor with the α TIF (viral transactivator protein) and the Oct-1 protein are critical to the assembly of the transcription complex, the domains of the C1 factor that mediate these interactions were biochemically mapped and characterized. These studies localized these domains to a small region of the protein and indicated that the factor interacted with both the viral transactivator and the POU-homeo domain of the Oct-1 protein. These studies will define biochemical interactions that are important for the regulated assembly of the transcription complex. [Kristie]

Proteolytic processing of C1. The novel proteolytic processing of the C1 factor at unique reiterations present in the protein indicated that an uncharacterized protease was involved. As the processing likely influences the regulation of C1 factor activity, genetic screens were designed to analyze the cleavage sequence and to isolate proteins that interact with these reiterations. These screens have resulted in the isolation of a number of characterized and novel cellular proteins that have indicated roles for the C1 factor in several basic cellular processes. [Vogel and Kristie]

Papillomavirus. The papillomaviruses are small DNA viruses that induce epithelial lesions in a wide range of vertebrates. Over 70 human viral types have been identified, and some have been associated with cervical carcinoma. The papillomavirus E1 and E2 proteins are important for initiation of viral DNA replication and for regulation of viral transcription and are potential targets for therapeutics.

Nuclear localization signals. The bovine papillomavirus type 1 (BPV-1) E2 transactivator and repressor proteins use different nuclear localization signals. The BPV-1 E2 gene encodes three polypeptides: the largest, E2-TA, is encoded by the entire open reading frame and functions as a transcriptional transactivator; two smaller polypeptides, E2-TR and EB/E2, are encoded by the 3' half of the reading frame and function as transcriptional repressors by antagonizing the function of E2-TA. All three proteins share a C-terminal DNA binding domain. A basic alpha helix within this domain that directly interacts with the DNA target can function as a nuclear localization signal; however, in the full-length E2-TA protein, the C-terminal nuclear localization signal is masked and a second signal, present in the N-terminal transactivation domain, is used for transport of the transactivator to the nucleus. The use of two different nuclear localization signals could potentially allow differential regulation of the subcellular localization of the E2 proteins. [Skiadopoulos and McBride]

E2 transactivator domains. The N-terminal domain of the BPV-1 E2 transactivator protein is important for viral DNA replication, for transcriptional transactivation, and for interaction with the E1 protein. To determine which residues of this 200 amino acid domain are important for these activities, single conservative amino acid substitutions were generated in residues that are invariant among all papillomavirus E2 proteins. Several mutated proteins were identified that were defective for either transcriptional activation, DNA replication, or the ability to co-operatively bind the replication origin with the E1 protein. A number of

mutated proteins that were wild-type for these activities were unable to transform cells, suggesting that the E2 transactivator may have additional functions in the viral life cycle. [Brokaw and McBride]

E1/E2 interactions. Transactivation and DNA binding domains of the BPV-1 E2 transactivator play distinct roles in co-operative binding to the replication origin with the E1 protein. The BPV-1 E2 transactivator protein binds co-operatively to the replication origin with the viral E1 protein. The replication origin contains an E1 binding site flanked by two E2 binding sites. To determine which regions and functions of the E2 protein are important for this interaction, a number of mutated E2 proteins were assayed for their ability to enhance E1 origin-specific binding. These studies demonstrated that the E2 transactivation domain is both necessary and sufficient for interaction with the E1 protein and that the E2 DNA binding domain is required for interaction with origin DNA sequences. The co-operative origin binding function of the E2 DNA binding domain can be replaced by heterologous DNA binding domains from other proteins, such as GALA. [Winokur and McBride]

Poxviruses. Unlike other DNA viruses, members of the poxvirus family replicate in the cytoplasm of the cell and encode many of the enzymes and factors needed for transcription of their genomes. Vaccinia virus, the best-studied member of this family, provides a unique system for combining biochemical and genetic approaches for investigating mechanisms of gene regulation and mRNA biosynthesis. Studies with vaccinia virus indicated that the genes are divided into three temporal classes—early, intermediate, and late—that are regulated in a cascade fashion. Our previous studies demonstrated that each class of genes has a unique promoter sequence and cognate transcription factors that work with a common, virus-encoded, DNA-dependent RNA polymerase. Efforts are being made to identify additional factors and to determine the mechanism of transcription and mRNA modification.

Ordered assembly of a functional preinitiation transcription complex, containing vaccinia virus early transcription factor and RNA polymerase, on an immobilized template. A functional pre-initiation transcription complex was formed by incubating vaccinia virus early transcription factor VETF and RNA polymerase with an early promoter template immobilized on paramagnetic particles. A preferred order of assembly, VETF followed by RNA polymerase, was demonstrated by stepwise addition experiments. The ability to form relatively stable preinitiation complexes in a stepwise manner should allow further characterization of the protein/DNA and protein/protein interactions involved. Such associations are likely to occur, at least partly, through the RNA polymerase-associated protein RAP94, which imparts early promoter specificity for the synthesis of RNAs as short as 4 nucleotides. [Cassetti and Moss]

Purification of intermediate transcription factors. Previous studies identified at least 3 components, in addition to RNA polymerase, required for intermediate transcription: capping enzyme, VLTF-1, and VLTF-2. Efforts are currently being made to complete the purification of VLTF-2. [Sanz and Moss]

Purification of new late transcription factors. Previous studies indicated a requirement for the vaccinia virus-encoded RNA polymerase and at least two additional factors for *in vitro* transcription of late stage promoters. An additional component, designated P3 factor, was isolated from vaccinia virus-infected cells. The factor was produced in the absence of viral DNA replication and was associated with the nuclear fraction. After DNA replication, the factor was found in both nuclear and cytoplasmic fractions. Our most highly purified preparations contain a single major band with an M_r of 35,000. In addition, a previously recognized transcriptional activator encoded by the A2L gene of vaccinia virus was shown to stimulate RNA synthesis in an *in vitro* assay and therefore probably has a direct role in transcription. [Kovacs, Passarelli, and Moss]

Mutational analysis of a multifunctional protein, with mRNA 5' cap-specific (nucleoside-2'-O-)-methyltransferase and 3'-adenylyltransferase stimulatory activities. The vaccinia virus-encoded protein VP39 is a poly(A) polymerase subunit that stimulates the formation of long poly(A) tails as well as a cap-specific mRNA (nucleoside-2'-O-)-methyltransferase. We have carried out mutagenesis studies aimed at locating regions of VP39 that are important for these activities. Truncation, charge cluster to Ala scanning, and Cys to Ser substitution mutations of VP39 were made, and the proteins were synthesized, purified, and analyzed. Deletion of the RNA binding domain, experimentally localized within the C-terminal 112 amino acids, resulted in loss of both mRNA modification activities. Eleven of the 21 charge cluster to Ala mutated proteins had low to nondetectable methyltransferase activity; four of those 11 also lacked adenylyltransferase stimulatory function. [Schnierle, Gershon, and Moss]

VIRAL DNA REPLICATION. Viruses are useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus-encoded factors provide potential targets for chemotherapy.

Herpesviruses. Several members of the herpesvirus family are significant human pathogens. These include herpes simplex virus (HSV), the best studied of the family (which causes painful skin lesions and occasionally causes life-threatening encephalitis), and Epstein-Barr virus (EBV), which causes infectious mononucleosis and is thought to be involved in some lymphocytic cancers. The study of herpesvirus DNA synthesis is useful as a model for eukaryotic DNA replication and for designing new antiviral strategies. Moreover, recent developments have suggested the possibility that following infection of neurons by HSV, lytic DNA replication may be a critical process that controls the establishment of latency. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication. Current efforts are directed toward studying this process with purified proteins, identifying required host proteins, investigating the role of DNA replication in latency, and extending the studies to the lytic replication of EBV.

Analysis of the components of the HSV-1 helicase-primase complex. To understand the contribution of UL52 to the helicase activity of the complex, we have constructed a number of

deletion mutants. These mutants are all defective for DNA replication, but the deleted polypeptides nevertheless interact with the other components of the complex. Further analysis of these mutants will be carried out to determine whether UL52 contributes structurally or catalytically to the helicase activity of UL5. We have also identified a number of amino acid insertion mutants of UL8 that are defective for DNA replication. These mutants will be used to assess the biological significance of certain *in vitro* biochemical activities of UL8. [Klinedinst, Fabisch, Lantz, and Challberg]

Characterization of the HSV primase. To understand the unique properties of the HSV primase more fully, we carried out experiments to determine whether the primase requires a specific template sequence for activity. Our results showed that, unlike other eukaryotic primases characterized to date, but similar to primases of certain prokaryotes, the HSV-1-encoded primase has a distinct preference for certain template sequences. The primase recognition site consists of a deoxy-guanosine followed (3'-5') by a stretch of at least five pyrimidines. The five pyrimidines can consist entirely of deoxycytidines, but not thymidines. [Gottlieb, Klindinst, and Challberg]

Role of the helicase primase in neurovirulence. To study the relationship between DNA replication and the outcome of infection of neurons, we have begun a project to analyze a strain of HSV in which a specific defect in DNA replication in neuronal cells has been traced to the presence of the UL5 gene from HSV-2. We have cloned this gene and its counterpart from wild-type HSV-2 and have introduced the genes back into wild-type HSV-1. In addition, we have constructed a baculovirus recombinant expressing this gene for biochemical analysis. [Barrera and Challberg]

Characterization of the origin binding protein. A number of studies have demonstrated that the HSV-1 UL9 protein, which is a homodimer in solution, binds to two high-affinity binding sites in each origin of replication. Interaction between the proteins bound at the two sites leads to the formation of a complex nucleoprotein structure. To understand this structure more fully, we have determined the stoichiometry of binding of the UL9 DNA binding domain to a single binding site. Unlike the full-length UL9 protein, the DNA binding domain is a monomer in solution. Our data show that two monomer units of the DNA binding domain bind to a single binding site. Interestingly, our data further suggest that the two monomer units in a dimer of the full-length protein cannot interact with the same site, so that it actually requires four monomer units of full-length protein to bind to one site, with two monomer units available for binding to a second site. The structural basis for this unusual mode of interaction is under investigation. [Fierer and Challberg]

Epstein Barr virus lytic DNA replication. Analysis of the structure of the EBV lytic origin and EBV genes required for lytic DNA replication suggests that the mechanism of initiation is likely to be unique. In particular, initiation depends not on a specialized viral initiator protein analogous to UL9, but on a key transcriptional transactivator protein (BZLF1). We have constructed most of the key reagents necessary for a biochemical analysis of EBV DNA replication studies, which consist primarily of recombinant baculoviruses expressing the

known EBV-encoded replication proteins. In initial studies, we have purified and characterized the single-stranded DNA binding protein, BALF2. We have been unable to obtain the helicase primase in soluble form and are attempting to develop an assay for EBV replication in insect cells in which the EBV replication proteins are provided by infection with the appropriate baculoviruses. [Zhen and Challberg]

Poxviruses. Poxviruses replicate in the cytoplasm of infected cells and encode proteins needed for DNA replication. One of these is a DNA polymerase.

Development of protein expression systems for altered forms of the vaccinia virus DNA polymerase. Several vaccinia viruses with a gene for an altered DNA polymerase have been isolated. To see what particular function of the DNA polymerase was affected by the alteration, attempts are being made to overexpress the enzyme in a vaccinia virus expression system designed in LVD, which is based on the use of a recombinant virus, vT7lacOI, that encodes the T7 RNA polymerase. To develop this procedure, the entire DNA polymerase gene was cloned in M13mp19 to produce a recombinant M13 phage which provides an abundant supply of a single strand of the wild-type polymerase gene. This DNA provides a template for genetic modification of the polymerase gene using an improved version of the Eckstein mutagenesis process. Basic modifications of the gene include removal of internal Nde I sites so that a unique Nde site could be positioned at the translation initiation codon. This construction which allows the polymerase sequence to be inserted into the Nde I site of the vaccinia transfer vector pVote2 places the inserted gene behind a T7 promoter and an EMC leader. PVote2 is designed to transfer the inserted DNA into the hemagglutinin region of the vT7lacOI vector which is the source of the T7 RNA polymerase. If this transfer occurs correctly, cells infected with the doubly recombinant virus should provide increased amounts of DNA polymerase so that it is convenient to purify altered forms of the enzyme. [DeFilippes]

VIRUS STRUCTURE, ASSEMBLY, AND CELL INTERACTIONS. Infection by enveloped viruses is initiated by binding of the viral envelope glycoprotein(s) to specific receptor molecules on the target cell, followed by fusion between the viral and cellular membranes. The study of viral envelope glycoprotein (env)/receptor interactions is an important area of research in LVD.

Human immunodeficiency virus (HIV). The human CD4 molecule has been shown to be important but not sufficient to mediate HIV infection and syncytium formation. One or more additional factors present in human but not nonhuman cells is critical. In addition, some strains of HIV are specific for lymphocytes, while others are specific for macrophages.

Human-specific accessory factor(s) for env glycoprotein/CD4 interactions. Using a recently developed reporter gene assay system to measure fusion between env-expressing and CD4-expressing cells, we have explored several aspects of HIV fusion: our previous results indicated that fusion requires the presence of a human-specific accessory factor(s) on the

CD4-expressing cell. We initiated molecular genetic approaches to identify this factor. Preliminary results indicate that murine cells expressing human CD4 can be rendered fusion competent by microinjection of mRNA from a human cell (HeLa) or by transfection with a HeLa cDNA library. Isolation of the active cDNA is in progress. [Broder, Feng, and Berger]

Cell type-specific accessory factors for HIV fusion. We previously demonstrated that the tropism of different HIV-1 isolates for infection of CD4-expressing T-cell lines vs. primary macrophages is associated with the intrinsic fusion specificities of the corresponding envelope proteins. Recent experiments indicate that a treatment of monocyte cell lines with differentiating agents renders them susceptible to fusion by envs from macrophage tropic isolates in parallel with acquisition of infection for such isolates. Experiments with transient cell hybrids reveal that T-cell line vs. macrophage tropism is associated with cell type-specific accessory fusion factors. Experiments are in progress to identify the accessory factor(s) associated with entry of macrophage-tropic isolates. [Broder, Alkhatib, and Berger]

Additional uses of the cell-cell fusion reporter gene assay. We have optimized conditions for the reporter gene assay to measure fusion inhibition by antibodies and pharmacological agents. [Kennedy and Berger]

Influenza virus. The major envelope glycoprotein of influenza virus, the hemagglutinin (HA), is the most extensively characterized viral envelope protein and has proven to be a useful model for studying similar proteins present on other viruses. Influenza remains a significant human pathogen, causing more mortality in the USA than any other virus. Since protection against influenza is provided nearly exclusively by the antibody response to the HA, it is essential to understand its structure to improve existing vaccines, which are only marginally effective.

Folding, assembly, and transport of influenza virus hemagglutinin. Monoclonal antibodies that distinguish between monomeric and trimeric forms of HA were used to localize the site of HA assembly in influenza virus-infected cells. This was achieved using a laser confocal scanning microscope to co-localize HA monomers and trimers with marker proteins that define subcompartments in the secretory pathway. The only compartment containing substantial amounts of HA monomers and trimers was the ER-Golgi intermediate compartment, which we propose to be the site of HA assembly. This conclusion was supported by examining the effects of drugs that perturb the early secretory pathway on the pattern of monomer and trimer staining. [Yewdell and Bennink]

Poxviruses. The formation of poxvirus particles is particularly complex, as there may be over 100 polypeptides in mature virions. Assembly begins with the formation of a membrane that is derived from the intermediate compartment between the endoplasmic reticulum and Golgi. The mature intracellular virions (IMV) are wrapped by a modified Golgi membrane prior to release from the cell as extracellular enveloped virions (EEV). We are in the process of identifying the viral protein components of the various membranes and determining their roles.

A myristylated membrane protein encoded by the vaccinia virus LIR open reading frame is the target of potent neutralizing monoclonal antibodies. We identified a protein component of the intracellular mature vaccinia virion membrane that is a target of a potent neutralizing monoclonal antibody. By immunofluorescent and electron microscopic analysis, the antibody was found to stain intracytoplasmic viral factories, virion membranes in cell sections. and the surface of negatively stained preparations of purified virions. The antigen, which is synthesized at late times in infection, has apparent molecular masses of 25 kDa and 29 kDa under nonreducing and reducing conditions, respectively. Although the N-terminus of the immunoaffinity-purified protein was blocked, sequence analysis of tryptic peptides revealed that the antigen was identical to the myristylated protein encoded by the LIR open reading frame. Validation of this genetic assignment was provided by immunoprecipitation of a [³H]myristic acid-labeled product of the expected molecular weight from infected cells.

Molluscum contagiosum virus (MCV). MCV is presently the only member of the poxvirus family that specifically infects humans. The skin lesions can persist for many months and are particularly troublesome in immunodeficient individuals including patients with AIDS. The object is to determine the organization of the molluscum contagiosum virus genome and to compare its mode of gene expression, replication, and host interaction with that of other poxviruses.

Analysis of the genome of molluscum contagiosum virus. A set of overlapping plasmids containing all but the very ends of the viral genome were sequenced. Preliminary analysis indicates that there are about 200 genes, of which approximately half are homologous to vaccinia and variola virus genes and the other half are unique to MCV. Many of the orthopoxvirus genes that are involved in host interactions and in formation of the outer membrane of the extracellular virus are not present in MCV. Some of the unique MCV genes are homologous to eukaryotic genes. [Koonina, Bugert, and Moss]

VIRAL IMMUNOLOGY. Virus infections are usually terminated following the development of effective humoral and cell-mediated immune responses by the host. An understanding of this process is important to understand how some viruses subvert the immune system and to develop effective vaccines or therapeutics.

Structure and function of antigenic peptide transporters. The efficient presentation of antigens to the immune system requires the expression of a transporter (TAP) that conveys peptides from the cytosol to the endoplasmic reticulum (ER). TAP comprises two subunits, termed TAP1 and TAP2. To analyze the structure and function of the transporters, recombinant vaccinia virus (rVVs) expressing TAP1 or TAP2 or both subunits were constructed. These rVVs express functional TAPs that allow cells missing one or both TAP subunits to present antigens to T_{CD8+}. Biochemical studies using the rVVs provide the initial characterization of the ATP binding of intact TAP1 and TAP2 subunits and have also established that the subunits assemble extremely rapidly following their synthesis. [Russ, Bennink, and Yewdell]

Class I molecule assembly and trafficking. Class I molecules of the major histocompatibility complex (MHC) bind peptides derived from cytosolic proteins and carry them to the cell surface for recognition by T cells. To study the assembly and trafficking of class I molecules, fluorescent peptides and metabolically labeled class I oligosaccharides are being used in TAP-deficient mutant cells and their wild-type parents. Similar amounts of class I molecules are detected in the Golgi complexes and are transported with similar kinetics to the surface of TAP-expressing and TAP-deficient cells; however, cells with TAP have up to six times more peptide-receptive class I molecules on their plasma membrane than TAP-deficient cells. This finding indicates 1) that many of the empty class I molecules reaching the cell surface of TAP-deficient cells are defective and either cannot bind peptides or are rapidly destroyed, or both, and 2) that most peptide-receptive molecules on normal cells arise from class I molecules that have lost their TAP-provided peptide. Using confocal microscopy to localize the binding of fluorescent peptides to fixed and permeabilized cells, we confirm that peptide-receptive class I molecules are present throughout the secretory pathways of normal and TAP-deficient cells. Extending these studies to live cells, we found that antigenic peptides are internalized from the culture medium to the endoplasmic reticulum, probably via a novel vesicular route. [Day, Bennink, and Yewdell]

Assembly, intracellular trafficking, and function of MHC class Ib molecules. Classical class I molecules present endogenous peptide antigens for recognition by cytotoxic T lymphocytes, which are transported by a heterodimer encoded by the *TAP1* and *TAP2* genes. Nonclassical class I (class Ib) molecules, although similar in structure to classical class I molecules, have an unknown function. We studied the cell biology and biochemistry of the representative murine class Ib molecules Q7^b and mCD1.1 with the ultimate goal of determining whether nonclassical class I molecules can present viral antigens to cytotoxic T cells. To study these proteins in a variety of tissue culture cell lines in a strictly controlled manner *in vitro* and in mice, we produced vaccinia virus recombinants expressing these proteins. Using these recombinants, we found that cell surface expression of both Q7^b and mCD1.1 requires assembly with β_2 -microglobulin. Q7^b expression also requires cells to express TAP, while mCD1.1 expression is TAP independent. We also found that the antigen presentation function of mCD1.1 to a unique subset of T cells parallels the requirement for surface expression. [Brutkiewicz, Bennink, and Yewdell]

Proteolytic processing of antigenic peptides in the endoplasmic reticulum. A critical question in antigen processing is whether antigenic peptides are trimmed to their final length after they have been imported into the endoplasmic reticulum (ER) from the cytosol. To explore this question, we created a number of vaccinia virus recombinants expressing proteins targeted to the ER that consist of antigenic peptides surrounded by different flanking sequences. Using cells dependent on ER processing for antigen presentation, we found that the ER is able to efficiently liberate the COOH terminal peptide, but not the NH₂-terminal peptide of a "tandem antigenic peptide." These findings indicate that the ER has endogenous peptide-trimming activity and may remove even very long amino terminal extensions following TAP-mediated translocation. While exploring the effect of inserting Asn-linked glycosylation sites in

antigenic peptides, we discovered that peptide precursors created in the ER may recycle to the cytosol for further trimming. [Link, Yewdell, and Bennink]

Increasing immunogenicity of viral antigens. CD8⁺ lymphocytes play an important role in host immunity to viruses and other intracellular parasites. In an effort to develop more efficient vaccines for eliciting CD8⁺ T cells, we have produced recombinant vaccinia viruses expressing proteins that do not require specialized antigen processing machinery. These recombinants can be more efficient in eliciting CD8⁺ T-cell responses than traditional recombinants expressing full-length gene products. In addition, these findings suggest that recombinant vaccines (including naked DNA vaccines) encoding ER-targeted peptides might be the optimal method for eliciting T_{CD8+} responses. We have studied why so few peptides in a given viral protein elicit CD8⁺ responses. Our findings suggest that the major limiting factors are, in rank order: 1) binding to class I molecules, 2) TCR repertoire, and 3) liberation of peptide by cytosolic proteases. [Bacik, Yewdell, and Bennink]

Antigenic implications of human immunodeficiency virus type 1 envelope quaternary structure: oligomer-specific and -sensitive monoclonal antibodies. We previously described a panel of monoclonal antibodies raised against soluble oligomeric HIV-1 envelope (env) glycoprotein. Further analysis of the monoclonal antibodies indicated that the majority reacted with conformational epitopes within the gp120 or gp41 subunits. The majority of those directed against gp41 preferentially reacted with oligomeric env, and some reacted only with the oligomer. In contrast, half the monoclonal antibodies directed against the gp120 subunit preferentially recognized monomeric env; only one reacted more strongly with oligomer, and none were oligomer-specific. The neutralizing ability of the monoclonal antibodies are currently being analyzed. [Earl, Broder, and Moss]

Limited virus replication following SIV challenge of macaques immunized with attenuated MVA vaccinia expressing SIVsm env and gag-pol. The highly attenuated and host-restricted modified vaccinia virus Ankra (MVA) strain was used as a vector to express the simian immunodeficiency virus (SIV) envelope and gag-polymerase genes. Rhesus macaques were immunized with the live recombinant viruses and then challenged with an uncloned, homologous, cell-free SIV. All immunized macaques exhibited a sustained, marked reduction of virus load in plasma, PBMCs, and lymph nodes compared with control animals. [Hirsch, Wyatt, and Moss]

Development of vaccinia virus expression vectors. Previous studies have demonstrated that vaccinia virus can be used as a safe, efficient, selectable, cloning and expression vector with a variety of applications for laboratory research, biotechnology, and medical treatment and prophylaxis.

Stringent chemical and thermal regulation of recombinant gene expression by vaccinia virus vectors in mammalian cells. We developed a stringently regulated expression system for mammalian cells that employs 1) RNA polymerase promoter and terminator elements of bacteriophage T7, 2) the lac repressor and operator of *Escherichia coli*, 3) the RNA

translational enhancer of encephalomyocarditis virus, and 4) the genetic background of vaccinia virus. In cells infected with the recombinant vaccinia virus, reporter β -galactosidase synthesis was induced at least 10,000- to 20,000-fold upon addition of isopropyl- β -D-thiogalactoside or by temperature elevation from 30 to 37°C using a new temperature-sensitive *lac* repressor. Regulated synthesis of the secreted and highly glycosylated HIV-1 envelope protein gp120 was also demonstrated. Yields of both proteins were approximately 2 mg/10⁸ cells in 24 hours. [Fuerst and Moss]

Escherichia coli β -glucuronidase, *GUS*, as a marker for recombinant vaccinia viruses. The relatively small (1.9 kb) *E. coli gusA* gene encoding β -glucuronidase (GUS) was shown to be useful as a reporter for the isolation of recombinant vaccinia viruses. The absence of cross-reactivity between GUS and β -galactosidase suggests that the two reporters may be used in succession for the production of recombinant viruses containing multiple foreign genes or simultaneously for gene expression studies. [Carroll and Moss]

Selection of recombinant vaccinia viruses on the basis of plaque formation. We developed a procedure for isolation of recombinant vaccinia viruses based solely on plaque formation without a requirement for specific cell lines, selective medium, or special staining. The system consists of two components: 1) a mutant non-plaque-forming virus and 2) a plasmid vector that, through homologous recombination, can simultaneously introduce a foreign gene and repair the mutation in the viral genome. The mutant virus contains a deletion of the *vp37* gene, encoding a 37-kDa protein component of the viral outer envelope, that is required for efficient viral spread on cell monolayers. The plasmid vector contains a functional *vp37*, a strong synthetic viral early/late promoter, unique restriction sites for gene insertion, and flanking segments of viral DNA for homologous recombination. Following infection and transfection of cells with the mutant virus and plasmid vector, respectively, recombinant viruses are identified and isolated by their ability to form plaques. [Blasco and Moss]

Attenuated vaccinia virus vectors. Modified vaccinia virus Ankara (MVA) is a replication-deficient, host range-restricted vaccinia virus that has been shown to be safe as a smallpox vaccine. We have shown that MVA recombinants make effective vaccines against influenza virus in a mouse model and against SIV in a monkey model. We also considered that MVA would make a safer vector for laboratory expression studies. We therefore constructed a recombinant MVA that expresses the bacteriophage T7 RNA polymerase gene in nonpermissive mammalian cell lines as well as in permissive chick embryo fibroblasts. When infected cells were transfected with a plasmid containing a target gene regulated by a T7 promoter, transient expression occurred. The level of expression was similar to that obtained with replicating strains of vaccinia virus, and in some cases was even higher. The rapid cytopathic effect accompanied by destruction of the cell monolayer seen with standard replicating strains of vaccinia virus was not observed with the MVA vector. Moreover, the extreme host range restriction of MVA vectors provide an added degree of personal safety in the laboratory. [Wyatt, Rozenblatt, and Moss]

MAJOR ADMINISTRATIVE CHANGES

Personnel Changes. After a thorough and thoughtful deliberation by the NIH Central Tenure Committee, Dr. Edward Berger was approved for tenure. The tenure action was based on strong evidence of independence and research accomplishments.

Dr. Ehud Katz (Hebrew University) is spending a sabbatical year in LVD. Young scientists who have come to LVD for postdoctoral training this year include Ghalib Alkhatib (Biotechnology Research Institute, Montreal), Shiv Prasad (University of Minnesota), Patrick Sanz (University of Montpellier II), Teri Shors (Arizona State University), Wataru Sugiura (NIH, Japan), and Jodi Vogel (University of Chicago).

A number of young scientists, having completed their training and obtained positions elsewhere: Jane Brokaw (U.S. Surgical Corp), Lawrence Carroll (U.S. Patent Office), Nicholas Harris (QBI Enterprises, Ltd., Israel), and Vel Karacostas (Eastern Virginia Medical School).

Honors, Awards, and Service.

Edward Berger was a speaker at the NIAID symposium on *Adhesion Molecules in HIV Infection: Fact or Fiction*; the NIH Research Festival; and the FASEB Conference on Viral, Bacterial, and Protozoan Pathogenesis. He was a Session Chairperson at the Workshop of NIH Intramural AIDS Targeted Antiviral Program and also at the NIGMS 8th Meeting on *Structure of AIDS-Related Systems and Applications to Targeted Drug Design*. Dr. Berger has served on the NIAID (Intramural) Technology Evaluation Advisory Committee since 1989. He received competitive funding from the NIH Intramural AIDS Targeted Antiviral Program and a grant from the American Medical Association.

Jack Bennink, the Associate Laboratory Chief, is an Associate Editor of *The Journal of Immunology*. He received the NIAID Merit Award for his scientific accomplishments. He was an invited speaker at the Keystone Symposia Conference on *Molecular Aspects of Viral Immunity in the session on Vaccine Design and Strategies*; and invited to preside as Session Chairperson on *Biochemical and Functional Parameters of Antigen Presentation and Recognition* at the Mid-Atlantic Immunobiology Conference. Dr. Bennink also presented lectures at the University of Chicago and the Hershey Medical Center.

Patricia Earl was invited to lecture at the Second National conference on *Human Retroviruses and Related Infections* and at the Keystone Symposium on *HIV Pathogenesis*. She received competitive funding from the NIH Intramural AIDS Targeted Antiviral Program jointly with Dr. Moss.

Thomas M. Kristie serves on the Advisory board of *The Journal of Biomedical Science* and is a faculty advisor for the NIH Postdoctoral Fellows Committee.

Alison McBride is on the Scientific Committee for the 15th International Papillomavirus Workshop.

Bernard Moss, a member of the National Academy of Sciences, received the 1994 ICN International Prize in Virology. He is President of the American Society for Virology and continues as Head of the WHO Center for Research on Virus Vectors, Editor of *Virology*, and a member of the editorial boards of *the Journal of Virology*, *AIDS Research and Human Retroviruses*, *Advances in Virus Research*, *Current Opinion in Biotechnology*, and the *NIH Catalyst*. He serves on the NIAID AIDS Vaccine Evaluation Committee, the NIAID Tenure and Promotion Committee, and the Panel to Assess the NIH Investment in Research on Gene Therapy. He was the DNA virus Division Lecturer at the annual meeting of the American Society for Microbiology and was an invited speaker at the Carlos Fernandez Tomas Meeting on Molecular Medicine in Mexico and at the 39th OHOLO Conference on *Vaccines: Novel Strategies in Design and Production* in Israel.

Jonathan Yewdell received competitive funding from the NIH Intramural Aids Targeted Antiviral Program. He was invited to lecture at the International Congress of Immunology held in San Francisco and at the *T Cells and Cytokines* symposium held in Oxford. He also presented seminars at MRC, London; the Mt. Sinai School of Medicine; M.G.H.; Dartmouth College; and the Universities of Minnesota, North Carolina, Toronto, and Zurich.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00126-22 LVD
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT Functional Analyses of Vaccinia Virus DNA		
PRINCIPAL INVESTIGATOR F. M. DeFilippes Research Physicist LVD, NIAID Others:		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Genetic Engineering Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Previous experiments have shown that substitutions of some amino acids in a conserved region of the vaccinia virus DNA polymerase are allowed. Vaccinia virus containing the altered polymerase formed plaques similar to those of wild-type virus. To ultimately identify what aspect of DNA polymerase function was associated with the structure of this particular conserved region, it is desirable to overexpress the enzyme. If modest amounts of functional enzyme can be expressed from a cloned gene, then the <i>in vivo</i> substitutions can be tested <i>in vitro</i> . Attempts to express substantial amounts of even a portion of the enzyme in bacteria were not successful. However, others have shown that a functional vaccinia virus DNA polymerase can be overexpressed in tissue culture cells infected with a recombinant vaccinia virus. To follow that lead and improve on that type of expression system, I am attempting to insert the polymerase gene into a new vaccinia virus vector that does not require an additional virus or plasmid to achieve overexpression. To accomplish this, I first inserted the entire gene into the DNA of the replicative form of M13mp19 bacteriophage. The resulting phage contains a recombinant DNA with one strand of the polymerase gene. This construct allows the application of an improved form of the Eckstein mutagenesis procedure in which the <i>in vitro</i> mutagenic manipulations can be completed in one day. After several manipulations to remove internal NdeI restriction enzyme target sites, a unique NdeI site was installed at the locus of the first codon of the polymerase. This construct was inserted into the vaccinia virus transfer vector pVote2, and the recombinant DNA was isolated. This DNA is designed to transfer the polymerase gene by homologous recombination into the hemagglutinin region of the vaccinia virus vector. If the recombination is successful and the resulting virus causes infected cells to express ten times more functional polymerase than is produced with wild-type virus infection, then the supply of altered polymerase should be sufficient to conveniently conduct <i>in vitro</i> enzyme assays.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00298-14 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	B. Moss	Chief	LVD, NIAID
Other Investigators:	L. Wyatt	Microbiologist	LVD, NIAID
	M. Carroll	Visiting Fellow	LVD, NIAID
	S. Rozenblatt	Visiting Scientist	LVD, NIAID

COOPERATING UNITS (if any)

MedImmune, Gaithersburg, MD

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.8

PROFESSIONAL:

2.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The cytoplasmic site of gene expression and use of virally encoded enzymes are distinguishing features of vaccinia virus and other poxvirus vector systems that probably accounts for their consistent ability to express foreign genes derived from a variety of prokaryotic, eukaryotic, and viral sources. This feature, together with their ability to stably integrate and package large amounts of additional DNA without loss of infectivity, their wide host range, and the development of methods for isolating recombinant viruses, account for their diverse use and popularity.

The formation and isolation of recombinant vaccinia viruses remains a time consuming process especially when several genes need to be introduced into the vaccinia virus genome. We developed two simple new ways of selecting and screening recombinant vaccinia viruses that are dependent on plaque formation and expression of β -glucuronidase, respectively. A new highly inducible recombinant vaccinia virus expression system that employs the *Escherichia coli lac* repressor and bacteriophage T7 RNA polymerase was developed. We have continued to explore the use of the highly attenuated and host restricted MVA strain of vaccinia virus as an expression vector because of the added safety. A recombinant MVA that expresses the T7 RNA polymerase gene was constructed for use as a transient expression vector in the laboratory. In addition, we have further evaluated the immunogenicity of a recombinant MVA that expresses the influenza virus hemagglutinin and nucleoprotein genes in a mouse model system.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00307-14 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	G. Kovacs	NRC Research Fellow	LVD, NIAID
	M. Cassetti	Special Volunteer	LVD, NIAID
	X. Hu	IRTA	LVD, NIAID
	L. Passarelli	IRTA	LVD, NIAID
	N. Harris	Visiting Associate	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

6.1

PROFESSIONAL:

4.9

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human (b) Human tissues X (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Poxviruses unlike other DNA viruses replicate in the cytoplasm of the cell and encode many of the enzymes and factors needed for transcription of their genomes. Vaccinia virus, therefore, provides a unique system for combining biochemical and genetic approaches for investigating mechanisms of gene regulation and mRNA biosynthesis. Studies with vaccinia virus indicated that the genes are divided into three temporal classes - early, intermediate and late - that are regulated in a cascade fashion.

Progress was made in determining the mechanism of formation of the transcription initiation complex. A functional pre-initiation transcription complex was formed by incubating vaccinia virus early transcription factor VETF and RAP94⁺ RNA polymerase with an early promoter template immobilized on paramagnetic particles. A preferred order of assembly, VETF followed by RNA polymerase, was demonstrated by stepwise addition experiments. ATP was unnecessary for pre-initiation transcription complex formation but divalent cations were required specifically for the association of RNA polymerase. The ability to form relatively stable pre-initiation complexes in a step-wise manner should allow further characterization of the protein/DNA and protein/protein interactions involved.

The vaccinia virus encoded protein VP39 is a poly(A) polymerase subunit, that stimulates the formation of long poly(A) tails, as well as a cap-specific mRNA (nucleoside-2'-O-)-methyltransferase. We have carried out mutagenesis studies aimed at locating regions of VP39 that are important for these activities. Truncation, charge cluster to Ala scanning, and Cys to Ser substitution mutations of VP39 were made and the proteins were synthesized, purified and analyzed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00416-12 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recombinant vaccines against Retroviruses Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	P. Earl	Microbiologist	LVD, NIAID
	V. Karacostas	IRTA	LVD, NIAID
	C. Broder	IRTA	LVD, NIAID
	A. Otteken	Special Volunteer	LVD, NIAID
	W. Sugiura	Special Volunteer	LVD, NIAID

COOPERATING UNITS (if any)

V. Hirsch, LID, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH

TOTAL STAFF YEARS:

4.6

PROFESSIONAL:

4.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human (b) Human tissues X (c) Neither

 (a1) Minors

 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS). At present there is no effective vaccine against this disease, and therapeutic agents provide only limited help. The objects of this project are to characterize HIV antigens, determine the targets of humoral and cell-mediated immunity, and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing genetic information of HIV and the closely related simian immunodeficiency virus (SIV). These recombinant viruses have been used to prepare purified proteins, make monoclonal antibodies, produce targets for cytotoxic T cells, study CD4-envelope interactions, and make candidate vaccines.

We have continued to characterize a large panel of monoclonal antibodies that were produced against a novel soluble oligomeric form of the HIV-1 envelope protein. Of 35 mAbs directed against gp41, 21 preferentially reacted with oligomeric env. A subset of these mAbs reacted only with env oligomers. In contrast, only 1 of 27 mAbs directed against the gp120 subunit reacted more strongly with env oligomers than with monomers, and none were oligomer-specific. However, 50% of anti-gp120 mAbs preferentially recognized monomeric env, suggesting that some epitopes in gp120 are partially masked or altered by intersubunit contacts in the native env oligomer.

The highly attenuated and host-restricted modified vaccinia virus Ankra (MVA) strain was used as a vector to express the simian immunodeficiency virus (SIV) envelope and gag-polymerase genes. Rhesus macaques were immunized with the live recombinant viruses and then challenged with an uncloned, homologous, cell free SIV. All immunized macaques exhibited a sustained, marked reduction of virus load in plasma, peripheral blood mononuclear cells, and lymph nodes compared to control animals.

19-17

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00445-11 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT

Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR

M. D. Challberg

Section Head

LVD, NIAID

Others:

D. Fierer

Medical Fellow

LVD, NIAID

J. Gottlieb

IRTA Fellow

LVD, NIAID

D. Klinedinst

IRTA Fellow

LVD, NIAID

W. Zhen

Visiting Associate

LVD, NIAID

I. Barrera

Special Volunteer

LVD, NIAID

COOPERATING UNITS (if any)

Department of Pharmacology, Johns Hopkins Medical School, Baltimore, MD.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

7.17

PROFESSIONAL:

5.17

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

We are engaged in analysis of the lytic replication of two human herpesviruses: herpes simplex virus (HSV) and Epstein-Barr virus (EBV). We are studying the purified protein products of the viral genes that participate in DNA replication in lytically-infected cells, using both biochemical and molecular genetic approaches to understand the function of these polypeptides in detail. Our recent results regarding HSV proteins can be summarized as follows: 1) UL9, the viral protein that initiates DNA replication, binds to its cognate binding site as a complex of two dimeric molecules; 2) The HSV primase, unlike any other known eukaryotic primases, but similar to several prokaryotic primases, has a requirement for a specific template sequence; and 3) The EBV BALF2 gene product is a single-stranded DNA binding protein that binds cooperatively to single-stranded DNA. Between 15 and 25 bases of single-stranded DNA are required for efficient binding.

In addition, we have the following work in progress. We have constructed a large number of deletion and insertion markers of two of the subunits of the HSV helicase-primase. These mutant polypeptides are being analyzed to provide information regarding the structure of the heterotrimeric helicase primase complex and to determine the biological significance of certain biochemical assays that require these proteins. We have also begun to analyse an intertypic recombinant virus (HSV-1 x HSV-2) that shows a profound specific defect in the ability to carry out DNA replication in neuronal cells. Genetic analysis of this virus has shown that the defect can be traced to the helicase gene derived from HSV-2. We have cloned the helicase gene from this virus, and a number of experiments are in progress to determine the underlying mechanism of its neuronal-specific defect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00538-08 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions of Human Immunodeficiency Virus with CD4 Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.A. Berger, Ph.D., Acting Head, Molecular Structure Section, LVD, NIAID

Others: C.C. Broder, Ph.D. IRTA Fellow LVD, NIAID

Y. Feng, Ph.D. NRC Associate LVD, NIAID

P.E. Kennedy Microbiologist LVD, NIAID

G. Alkhatib Visiting Fellow LVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Diseases, NIAID, NIH

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.2

PROFESSIONAL:

3.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Infection by enveloped viruses is initiated by binding of the viral envelope glycoprotein(s) to specific receptor molecules on the target cell, followed by fusion between the viral and cellular membranes. We have been studying various aspects of viral envelope glycoprotein (env)/receptor interactions: Human immunodeficiency virus (HIV) env glycoprotein/CD4 interactions. Using a recently developed reporter gene assay system to measure fusion between env-expressing and CD4-expressing cells, we have explored several aspects of HIV fusion: a) Our previous results indicated that fusion requires the presence of a human-specific accessory factor(s) on the CD4-expressing cell. We initiated molecular genetic approaches to identify this factor. Preliminary results indicate that murine cells expressing human CD4 can be rendered fusion-competent by microinjection of mRNA from a human cell (HeLa), or by transfection with a HeLa cDNA library. Isolation of the active cDNA is in progress. b) We previously demonstrated that the tropism of different HIV-1 isolates for infection of CD4-expressing T-cell lines vs. primary macrophages is associated with the intrinsic fusion specificities of the corresponding envs. Recent experiments indicate that treatment of monocyte cell line with differentiating agents renders them susceptible to fusion by envs from macrophage tropic isolates, in parallel with acquisition of infection for such isolates. Experiments with transient cell hybrids reveal that T-cell line vs. macrophage tropism is associated with cell type-specific accessory fusion factors. Experiments are in progress to identify the accessory factor(s) associated with entry of macrophage-tropic isolates. c) We have optimized conditions for the reporter gene assay to measure fusion inhibition by antibodies and pharmacological agents.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00539-08 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Virus-Host Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	E. Wolffe	IRTA	LVD, NIAID
	R. Roper	IRTA	LVD, NIAID
	A. Ramsey-Ewing	IRTA	LVD, NIAID
	A. Grunhaus	IRTA	LVD, NIAID

COOPERATING UNITS (if any)

G. Griffiths, EMBL, Heidelberg; T. Pollard, Johns Hopkins University

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH

TOTAL STAFF YEARS:

4.6

PROFESSIONAL:

4.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

® (a) Human (b) Human tissues X (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A successful infection involves virus entry into the cell; uncoating, expression, and replication of the genome; assembly and release of infectious virus particles; and defense against specific and non-specific host immune mechanisms. Combined genetic, biochemical, electron microscopic, and immunologic approaches are being used to investigate these complex processes.

During the past year we continued work on the assembly of vaccinia virus. We identified a protein component of the intracellular mature vaccinia virion membrane that is a target of a potent neutralizing monoclonal antibody. By immunofluorescent and electron microscopic analysis, the antibody was found to stain intracytoplasmic viral factories, virion membranes in cell sections and the surface of negatively stained purified virions. The antigen, which is synthesized at late times in infection, has apparent molecular masses of 25 kDa and 29 kDa under non-reducing and reducing conditions, respectively. Although the N-terminus of the immunoaffinity purified protein was blocked, sequence analysis of tryptic peptides revealed that the antigen was identical to the myristylated protein encoded by the LIR open reading frame. Validation of this genetic assignment was provided by the ability of the antibody to immunoprecipitate a [3H]myristic acid-labeled product of the expected molecular weight from infected cells.

We expressed in *Escherichia coli* the vaccinia virus gene for a protein similar to vertebrate profilins, purified the recombinant viral profilin, and characterized it. The viral profilin differed from the vertebrate homolog by having a higher affinity for polyphosphoinositides than actin suggesting that it may have a primary role in phosphoinositide metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00541-08 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Folding, Assembly, and Transport of Viral Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID

COOPERATING UNITS (if any)

T. Bachi, Electron Microscopy Laboratory, Zurich, Switzerland;
H-P. Hauri, Biozenter, Basle, Switzerland.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.20 staff years

PROFESSIONAL:

0.10 staff years

OTHER:

0.10 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The improvement of current antiviral vaccines and the development of novel vaccines depends on increasing our understanding of viral attachment and fusion glycoproteins. Critical insight into understanding the antigenic structure of glycoproteins is provided by studying their interaction with monoclonal antibodies (mAbs). For a number of years we have studied the influenza virus hemagglutinin (HA) glycoprotein. This protein serves as a model for other proteins with similar functions (e.g., HIV gp160), and moreover, is important practically in its own right, as influenza still is a major cause of morbidity and mortality nationally, and internationally. Like many viral glycoproteins the HA is a homo-oligomer, consisting of three identical monomeric subunits. In the past year we continued to investigate the site of trimerization of newly synthesized HA. Our previously published findings suggested that HA trimerization occurs only after monomers are exported from the ER. In the past year we have used a number of novel inhibitors of ER to Golgi complex traffic to demonstrate immunocytochemically and biochemically that trimerization occurs in the ERGIC (acronymic for ER-Golgi Intermediate Compartment).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00542-08 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Viral Proteins for T Cell Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID
Others: Patricia Day	IRTA Fellow	LVD, NIAID
Shiv Prasad	IRTA Fellow	LVD, NIAID

COOPERATING UNITS (if any)

Jan Lukso, BRB, NIAID;
Ajit Varki, UCSD Cancer Center, San Diego, CA.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.40 staff years

PROFESSIONAL:

2.20 staff years

OTHER:

0.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class I molecules of the major histocompatibility complex (MHC) consist of a highly polymorphic heavy chain complexed to β_2 microglobulin (β_2m). Class I molecules are constitutively expressed by numerous cell types in the body. Expression by non-expressing cell types occurs rapidly in the course of an immune response following exposure to γ -IFN and other cytokines. The sole function of class I molecules is to bind antigens and present them to T cell bearing CD8 molecules (T_{CD8+}). T_{CD8+} play a critical role in eradicating intracellular pathogens and tumors. They can also contribute to immunopathology, being involved in organ rejection and autoimmune diseases. There has been rapid progress in understanding the physical nature of the antigen-class I complex, and in how antigens are generated and become associated with class I molecules in cells. Peptides of 8 to 15 residues produced from a cytosolic pool of proteins by cytosolic proteases are translocated into the endoplasmic reticulum (ER) by a MHC encoded transporter complex known as TAP. Once in the ER, peptides (possibly after further trimming by peptidases) bind to class I molecules associated with TAP and are transported to the cell surface. In the past year we have continued our studies the assembly and trafficking of MHC class I molecules and have made progress on a number of fronts:

- 1) We developed fluorescent derivatives of an antigenic peptides and used these probes to make several novel findings about the intracellular trafficking of class I molecules and antigenic peptides,
- 2) We have studied the intracellular trafficking of class I molecules using [3H]-labeled saccharides to label class I associated N-linked oligosaccharides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00619-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intracellular Antibody-Mediated Virus Neutralization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Bennink Microbiologist LVD, NIAID
 J.W. Yewdell Medical Officer (Research) LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.0 staff years

PROFESSIONAL:

0.0 staff years

OTHER:

0.0 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of the greatest hurdles in creating vaccines for a number of viral pathogens is the antigenic variability of viral coat proteins. The rapid mutation rate of a number of viruses (most notably influenza virus and HIV) allows the virus to escape the neutralizing antibody response induced by anti-viral vaccines. The internal proteins of the virus, on the other hand, and generally highly conserved, and have not evolved to rapidly alter their antigenicity. Antibody responses to these internal proteins, while a consistent feature of immune responses to viruses, almost always fail to influence viral infectivity, since the antibodies do not access to their target antigens, which are located either inside the virus, or inside the virus infected cells. If, however, antibodies are introduced into the cytosol of cells, they do have the ability to prevent viral infection. Recent advances in understanding antibody folding indicate that antibodies can under some circumstances properly fold and bind antigen when they are expressed in the cytosol by removing their amino terminal ER insertion sequences. Therefore, cells, or eventually, transgenic animals expressing such cytosolic antibodies should be resistant to virus infection. Using electroporation to introduce antibodies into the cytosol, we identified antibodies able to block infection of cells with influenza A or B viruses. Our ultimate goal is to clone the genes encoding these antibodies and express them in the cytosol of tissue culture cells, and eventually transgenic animals, thus creating animals resistant to influenza virus infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00652-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Peptide Transporters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.W. Yewdell Medical Officer (Research) LVD, NIAID

J.R. Bennink Microbiologist LVD, NIAID

Others: Gustav Russ Visiting Associate LVD, NIAID

COOPERATING UNITS (if any)

P. Cresswell, Yale University, New Haven, CT

D. Johnson, McMaster Univ. Hamilton, Ontario, Canada

H. Ploegh, MIT, Cambridge, MA; K. Parker, JMS

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.40 staff years

PROFESSIONAL:

1.20 staff years

OTHER:

0.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

CD8+ T cells (T_{CD8+}) play an important role in controlling virus infections. T_{CD8+} recognize peptides of 8 to 10 residues derived from viral proteins located in the cytosol of virus infected cells. These peptides are recognized in a complex with class I molecules encoded by the major histocompatibility complex (MHC). In the past 3 years it was discovered that the MHC also encodes two molecules, termed TAP1 and TAP2, that combine in a 1:1 ratio to create a complex that specifically transports peptides from the cytosol into the endoplasmic reticulum (ER). Human TAP genes display at least some limited polymorphism. The existence of these peptide pumps and their polymorphism raises a number of important questions: Where in the cells are the pumps located? Do the pumps influence the types of peptides presented by class I molecules? Are the pumps tethered to the proteases that produce antigenic peptides in the cytosol? How do the pumps work? Are there individuals with immune deficiencies based on mutations in the TAP genes? Can cells transport peptides via other mechanisms? To help characterize the structure and function of the TAP genes we have created recombinant vaccinia viruses (rVV) that express either TAP1 (VV-TAP1_h), TAP2 (VV-TAP2_h), or TAP1 and TAP2 (VV-TAP[1&2]_h). We have demonstrated that each of rVVs express functional subunits, or in the case of the VV-TAP[1&2]_h, a functional transporter. Using this panel of rVVs we have studied the assembly, intracellular location, and biochemical properties of TAP1 and TAP2, and in collaboration with other laboratories, demonstrated that a herpesvirus protein binds human TAP and blocks its function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00653-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Delivery of Antigens to the MHC Class I Processing Pathway

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Bennink	Microbiologist	LVD, NIAID
J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others: I. Bacik	Visiting Fellow	LVD, NIAID
Y. Deng	IRTA Fellow	LVD, NIAID

COOPERATING UNITS (if any)

B. Murphy, LID, NIAID, NIH; N. Restifo, Surgery Branch, NCI, NIH;
S. Tevethia, Hershey Medical Center, Hershey, PA;
V. Nussenweig, New York University, New York, NY.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.40 staff years

PROFESSIONAL:

1.20 staff years

OTHER:

.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

CD8 positive lymphocytes (T_{CD8+}) play an important role in host immunity to viruses and other intracellular parasites. Antiviral T_{CD8+} recognize MHC class I molecules bound to peptides derived from a cytosolic pool of viral proteins. The induction of antiviral T_{CD8+} responses is potentially limited by the rates at which peptides are generated from full-length gene products, and the rate at which peptides are transported into the ER by TAP, the MHC-encoded peptide transporter. In an effort to develop more efficient vaccines for eliciting T_{CD8+} we created a number of recombinant vaccinia viruses that express antigenic peptides in the absence of flanking residues, or with an amino terminal extension that targets the peptides to the endoplasmic reticulum. Testing of these recombinants indicates that these recombinants more efficiently elicit T_{CD8+} responses than traditional recombinants expressing full length gene products.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00658-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Proteolytic Generation of Antigenic Peptide

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Bennink	Microbiologist	LVD, NIAID
J.W. Jewdell	Medical Officer (Research)	LVD, NIAID

Others:

Luis Anton	Visiting Fellow	LVD, NIAID
Igor Bacik	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

Alex Vinitsky, Mt. Sinai School of Medicine, NY, NY
Martin Rechsteiner, University of Utah, Salt Lake City, UT
Clive Slaughter, University of Texas Southwestern Medical Center, Dallas, TX

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.60 staff years

PROFESSIONAL:

1.40 staff years

OTHER:

.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

CD8 expressing T cells (T_{CD8+}) play a critical role in eradicating intracellular parasites such as viruses. T_{CD8+} recognize MHC class I molecules in a complex with peptides of 8 to 10 residues derived from viral proteins located in the cytosol. There is tremendous interest in the mechanism by which peptides are generated. There is indirect evidence that implicates proteasomes in the generation of antigenic peptides. Proteasomes are abundant macromolecular structures present in the cytosol and nucleus in cells, and have multiple protease activities. They are thought to be responsible for energy dependent proteolysis in which ubiquitin plays a prominent role in targeting proteins for destruction. Although it is believed that at least some proteolysis occurs in the cytosol, it is uncertain whether the ultimate determinants are generated in the cytosol or whether additional trimming occurs following transport from the cytosol. We have initiated several approaches to assess the site and nature of proteolytic mechanisms utilized in the generation of antigenic peptides. First, have studied antigen processing in cells defective in their capacity to ubiquitinate proteins. Second, we have examined the relationship between metabolic stability of a protein and its efficiency as a source of antigenic peptides. Third, we examined the requirement for MHC encoded proteasome subunits in antigen processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00659-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Presentation by Human Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Bennink Microbiologist LVD, NIAID
J.W. Yewdell Medical Officer (Research) LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.20 staff years

PROFESSIONAL:

0.10 staff years

OTHER:

0.10 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is a considerable body of evidence that tumor-specific CD8⁺ T lymphocytes (T_{CD8+}) can be elicited in tumor-bearing individuals and can mediate a significant antitumor effect. In most cases, however, tumor-specific T_{CD8+} do not arise naturally. We are investigating the possibility that the poor immunogenicity of some tumors is due to limitations in the abilities of cancer cells to present their tumor-specific antigens to the immune system. In the past few years there has been tremendous progress in our understanding of how cells present antigens for recognition by T_{CD8+}. It is now clear that presentation of antigen results from a chain of events, including protein breakdown into peptides of 8 to 10 amino acids, transport of peptides from the cytosol into the endoplasmic reticulum, binding of peptides to a special peptide-presenting molecule, and delivery of the peptide complexed-presenting molecule to the cell surface. To test the hypothesis that limitations in tumor antigen breakdown or transport of the antigen into the endoplasmic reticulum limit the immunogenicity of T cells, we have created novel vaccines in which antigenic peptide generation and transport occurs independently of the normal antigen processing machinery. We are currently testing whether such vaccines are able to elicit tumor-specific T_{CD8+} and can protect animals against a challenge with tumor cells or cure animals with existing tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00660-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Brefeldin A Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.W. Yewdell Medical Officer (Research) LVD, NIAID
J.R. Bennink Microbiologist LVD, NIAID

Others: Yuping Deng Visiting Fellow LVD, NIAID

COOPERATING UNITS (if any)

R. Haugland and H.C. Kang, Molecular Probes, Eugene, OR.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.30 staff years

PROFESSIONAL:

0.20 staff years

OTHER:

0.10 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brefeldin A (BFA) is a fungal metabolite with a broad range of biological activities, including antifungal and antitumor activities. In the past few years there has been tremendous interest in its effects on cells, since it has been found to have a unique ability to interfere with vesicular trafficking in cells. Among its effects, it disperses Golgi complex, returning at least some of its components to the endoplasmic reticulum (ER). BFA also prevents newly synthesized proteins destined for the cell surface from leaving the ER. This property is responsible for its ability to completely block the presentation of viral antigens to cytotoxic T lymphocytes. To better understand the mechanism of BFA action, we conjugated it to two fluorescent dyes and studied its intracellular localization. Both conjugates maintained their biological activity, and both specifically localized to the ER and Golgi complex in live or aldehyde-fixed cells. Localization is specific since unconjugated dye, or dye conjugated to a molecule of similar structure to BFA do not exhibit the same pattern of localization. The selective partitioning of conjugated BFA into intracellular membranes is probably due to its interaction with lipids as it is abolished by detergent extraction of lipids. These conjugates are the first dyes that bind the ER and Golgi complex without binding other prominent membrane bound compartments, and should prove useful as probes for these organelles in living cells. The interaction of BFA with the ER and Golgi complex membranes may be essential to its effects on vesicular trafficking.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00661-04 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Confocal Microscopy of Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID

COOPERATING UNITS (if any)

K. Kelly, LP, DCBD, National Cancer Institute, NIH;
 L. Staudt, MET, National Cancer Institute, NIH

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.40 staff years

PROFESSIONAL:

0.20 staff years

OTHER:

0.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Recent advances in optics and electronics have culminated in the development of the laser confocal scanning microscope (LCSM). The LCSM provides unparalleled resolution for localizing fluorescent probes in cells. In combination with advanced—yet relatively inexpensive and compact—computers, it is relatively easy to produce 3-dimensional reconstructions of fluorescently labeled structures in cells. We have been working with a state-of-the-art BioRad MRC 600 LCSM with its controlling computer. To produce 3-D reconstructions of data sets obtained with the LCSM, we use VoxelView software on a Silicon Graphics Iris Indigo computer. We have used this system for a wide variety of applications, including determining the subcellular distribution of newly defined proteins and following the intracellular trafficking of newly synthesized membrane proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00690-03 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Processing in Lower Eukaryotic Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.W. Yewdell

Medical Officer (Research)

LVD, NIAID

J.R. Bennink

Microbiologist

LVD, NIAID

Others: Yuping Deng

Visiting Fellow

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Cellular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.80 staff years

PROFESSIONAL:

0.60 staff years

OTHER:

0.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

CD8⁺ T cells recognize class I molecules of the major histocompatibility complex (MHC) bearing peptides of 8 to 10 residues derived from cytosolic proteins. These cells are a bulwark of host defenses to infectious agents and tumors, and if we are to improve existing vaccines and develop new vaccines and treatments for infectious and neoplastic diseases, it is critical to understand antigen processing, the mechanism by which antigenic peptides are generated by cells and delivered to class I molecules. Although there has been great progress in understanding antigen processing in the past 5 years, there remains much to be learned. The strategy of this project is to delineate what we do not know. We are expressing the known constituents of the antigen processing machinery in mosquito cells and determining whether this is sufficient to reconstitute antigen processing to a level seen in mammalian cells. Insects do not have a MHC and do not, therefore, have any of the specialized machinery associated with antigen processing. If these cells can process antigens under these conditions, this would indicate that we have identified the major components of antigen processing; if they cannot, it would mean that further discoveries are required. In the past year, we have found that class I molecules expressed in cells derived from an invertebrate fail to properly assemble with peptides delivered to the ER, suggesting the existence of accessory molecules in higher eukaryotic cells that load peptides onto class I molecules.

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Proteolytic Processing of Antigenic Peptides in the Endoplasmic Reticulum

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Bennink	Microbiologist	LVD, NIAID
J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others:		
Heidi Link	IRTA Fellow	LVD, NIAID
Igor Bacik	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.70 staff years

PROFESSIONAL:

1.50 staff years

OTHER:

0.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

CD8⁺ T cells (T_{CD8+}) recognize class I molecules of the major histocompatibility complex (MHC) bearing peptides of 8 to 10 residues derived from cytosolic proteins. These cells are a bulwark of host defenses to infectious agents and tumors, and it is critical to understand how antigenic peptides are generated by cells if we are to improve existing vaccines and develop new vaccines and treatments for infectious and neoplastic diseases. Very little is known about how cells produce antigenic peptides from proteins. While it is clear that proteolytic production of peptides begins in the cytosol, it is uncertain to what extent trimming occurs after peptides are translocated into the endoplasmic reticulum (ER). Previous results from our laboratory indicate that trimming of peptides in the ER can occur under the special circumstances of expressing an exotic secretory protease in antigen presenting cells. To extend these findings, we have used a novel strategy to characterize the endogenous proteolytic capacity of the ER and its relevance to antigen processing. Our findings demonstrate that ER proteases, most likely aminopeptidases, are able to liberate antigenic peptides from longer precursors. In conjunction with recent findings from other laboratories, our results suggest a model in which peptides from cytosolic proteins are transported into the ER with amino terminal extensions that are removed by an aminopeptidase residing in the ER.

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of a Novel Mammalian Transcription Factor Involved in Enhancer Complex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T. Kristie Investigator LVD, NIAID

J. Vogel IRTA LVD, NIAID

R. Dashner Microbiologist LVD, NIAID

COOPERATING UNITS (if any)

A. Sears, Department of Microbiology and Immunology, Emory University, Atlanta, GA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892-0455

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Expression of the immediate early genes of herpes simplex virus I requires the assembly of a multiprotein RNAPII enhancer complex consisting of several viral and cellular transcription factors. This assembly provides a model for the analysis of the components involved in the specific activation of gene expression. The present study focuses upon the characterization of the mammalian C1 factor; a protein complex which interacts with the viral transactivator and is required for the enhancer assembly.

Analysis of isolated cDNAs encoding the C1 factor indicate that it is a novel family of proteins which are specifically proteolytically processed from a large precursor. These proteins are generated by site-specific cleavage within a reiterated amino acid sequence. As the processing and assembly of this factor influences its activity, genetic screens have been designed for the analysis of the cleavage sequences and the isolation of the specific protease from mammalian cells. These studies have resulted in the identification of a number of novel factors which interact with the reiterated sequences of the C1 factor.

The development of specific antiseras have determined that although the C1 factor is ubiquitously expressed in most tissues, the protein is localized to specific subnuclear structures in certain cell types. In addition, C1 factor homologs and have been identified and isolated from other animal systems to allow for the genetic analysis of these factors.

The continuing studies focus upon questions concerning the role of the C1 factor processing in the transcription of the herpes simplex virus genes, the specific transport/localization of the protein, and the development of animal systems for the analysis of the functions of the protein in viral and cellular functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00712-02 LVD
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT Regulation of RNAPII-Directed Transcription of Herpes Simplex Virus		
PRINCIPAL INVESTIGATOR <div style="display: flex; justify-content: space-between; margin-top: 5px;"> T. Kristie Microbiologist LVD, NIAID </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> Others: R. Dashner Microbiologist LVD, NIAID </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Genetics Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: .5	PROFESSIONAL: .5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Transcription of eukaryotic genes by RNAPII requires the assembly of multiple protein factors into a transcription initiation complex. In addition, the regulation of this process with respect to the selective activation of specific genes and the rate at which these genes are transcribed is often dependent upon interactions of protein regulatory factors with this complex.</p> <p>Herpes simplex virus is used as a model system for the analysis of the genetic sequences and protein factors required for the regulated transcription of mammalian genes by RNAPII. In the course of a viral infection, three classes of genes (Immediate early, Early, and Late) are transcribed in a distinct temporally regulated manner. Of these, the Immediate early genes are regulated by the interactions of viral (TIF) and host cell proteins (Oct-1 and C1 factor). Several approaches have been used to identify and analyze the domains and interactions of these factors which are required to regulate the initiation of IE gene expression. The continuing study is involved in the development of an in vitro transcription system which reproduces the regulatory requirements of each viral gene class. This will allow for the analysis of the basic and specific protein factors which selectively activate a particular gene class as well as elucidation of the biochemical mechanisms involved in this process.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00713-02 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT

Characterization of the Papillomavirus Regulatory Proteins

PRINCIPAL INVESTIGATOR

P.I.:	A.A. McBride	Investigator	LVD, NIAID
Others:	J.L. Brokaw	IRTA Fellow	LVD, NIAID
	M.H. Skiadopoulos	IRTA Fellow	LVD, NIAID
	M. Blanco	Biologist	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.25

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The papillomaviruses induce epithelial lesions in a wide range of vertebrates and over 70 human viral types have been identified. Although these lesions are normally benign, they can progress to malignant carcinomas, such as cervical cancer. The E1 and E2 proteins of the papillomaviruses regulate gene expression and DNA replication of the virus. Because the E1 and E2 proteins have both positive and negative effects on the viral life cycle, a detailed understanding of their regulatory mechanisms is crucial for the design of antiviral drugs and strategies. In the past year, we have determined that the transactivation and DNA binding domains of the BPV-1 E2 transactivator play distinct roles in co-operative binding to the replication origin with the E1 protein. The E2 transactivation domain is necessary and sufficient for interaction with the E1 protein and the E2 DNA binding domain is required for interaction with origin DNA sequences. The function of the DNA binding domain can be replaced by heterologous DNA binding domains from other proteins. By generating conservative amino acid substitutions in residues that are invariant among all papillomavirus E2 proteins, we have identified amino acids in the E2 transactivation domain that are critical for transcriptional activation, for DNA replication, and for interaction with the E1 protein. Several viral genomes that encode mutated E2 proteins that are wild-type for transactivation, replication, and interaction with the E1 protein are defective in cellular transformation, suggesting that the E2 proteins have additional functions that are important for the viral life cycle. We have also discovered that the BPV-1 E2 transactivator and repressor proteins use different nuclear localization signals. Both proteins share a common C-terminal DNA binding domain and a basic region within this domain forms an alpha helix that makes direct contact with the DNA target. This basic region functions as a nuclear localization signal, both in the E2 C-terminal domain and in a heterologous protein; however, in the full-length E2 transactivator protein, the C-terminal NLS appears to be masked and a second signal, present in the N-terminal transactivation domain, is used for transport of the transactivator to the nucleus. The use of two different nuclear localization signals could potentially allow differential regulation of the subcellular localization of the E2 proteins at some stage in the viral life cycle.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00714-02 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mollusum Contgiosum Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	J. Bugert	Special Volunteer/IRTA	LVD, NIAID
	T. Koonina	Visiting Associate	LVD, NIAID

COOPERATING UNITS (if any)

G. Darai, University of Heidelberg

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human x (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mollusum contagiosum virus is a human poxvirus that causes lesions that can persist for months to years in the skin of healthy or immunodeficient individuals, particularly children. Infection with this virus is increasing and has become a troublesome complication for adults with AIDS. At present there is no specific preventive or treatment for the disease. All attempts to culture the virus have failed and little is known about it. The object of the project is to analyze the structure of the viral genome and determine its mode of expression and replication. The way in which the virus resists the human immune system is of particular interest.

A set of overlapping plasmids containing all but the very ends of the viral genome were sequenced and the data are being assembled. Preliminary analysis indicates that there are about 200 genes of which approximately half are homologous to vaccinia and variola virus genes and the other half are unique to mollusum contagiosum virus. Many of the orthopoxvirus genes that are involved in host interactions are not present in mollusum contagiosum virus. Some of the unique mollusum contagiosum virus genes are homologous to eukaryotic genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00731-01 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assembly, intracellular trafficking, and function of MHC class Ib

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
J.R. Bennink	Microbiologist	LVD, NIAID

Others:	Randy Brutkiewicz	NRC Fellow	LVD, NIAID
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COOPERATING UNITS (if any)

Albert Bendelac, Princeton University, Princeton, NJ
 Steven Balk, Brighams and Women's Hospital, Boston, MA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.40 staff years

PROFESSIONAL:

1.20 staff years

OTHER:

0.20 staff years

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Classical class I molecules present endogenous peptide antigens for recognition by cytotoxic T lymphocytes, which are transported by a heterodimer encoded by the *TAP1* and *TAP2* genes. Nonclassical class I (class Ib) molecules, although similar in structure to classical class I molecules, have an unknown function. We are interested in studying the cell biology and biochemistry of the representative murine class Ib molecules, Q7^b and mCD1.1, with the ultimate goal of determining whether nonclassical class I molecules can present viral antigens to cytotoxic T cells. To study these proteins in a variety of tissue culture cell lines in a strictly controlled manner in vitro and in mice we produced vaccinia virus recombinants expressing these proteins. Using these recombinants we found that cell surface expression of both Q7^b and mCD1.1 requires assembly with β_2 -microglobulin. Q7^b expression also requires cells to express TAP, while mCD1.1 expression is TAP independent. In collaboration with A. Bendelac (Princeton University) we found the antigen presentation function of mCD1.1 to a unique subset of T cells parallels the requirement for surface expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI-00733-01 LVD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Paramyxovirus Glycoproteins/Receptor Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.A. Berger, Acting Head, Molecular Structure Section, LVD, NIAID

Others: P.E. Kennedy, Microbiologist, LVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20802

TOTAL STAFF YEARS:

.4

PROFESSIONAL:

.2

OTHER:

o.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Infection by enveloped viruses is initiated by binding of the viral envelope glycoprotein(s) to specific receptor molecules on the target cells, followed by fusion between the viral and cellular membranes. We have initiated mechanistic studies of fusion mediated by glycoproteins of paramyxoviruses: SV5, measles virus (MV), and canine distemper virus (CDV). In all cases cell fusion requires co-expression of the virus fusion (F) and hemagglutinin (HA or HN) glycoproteins on the surface of one cell, as well as the presence of the appropriate receptors on the other cell. For the morbillaviruses MV and CDV, fusion occurs efficiently with heterologous expression of F from one virus and H from the other; cell-type specificity is determined by H. We also obtained direct evidence for functional and structural interaction between measles H and CD46, the known MV receptor.

ROCKY MOUNTAIN LABORATORIES MICROSCOPY BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1995 Annual Report
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ANNUAL REPORT
ROCKY MOUNTAIN LABORATORIES MICROSCOPY BRANCH
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1994, TO SEPTEMBER 30, 1995

The Rocky Mountain Laboratories Microscopy Branch (RMMB) has both a well developed research program and a core facility function. As part of its research function, the Branch investigates the molecular structure and function of genes and gene products that are important in bacterial pathogenesis. Although a multidisciplinary approach is used to study aspects of bacterial infection and disease, important human pathogens such as those causing enteric disease, tuberculosis and Lyme disease are emphasized. The Branch's research laboratories use modern molecular biology methods to study bacterial pathogens and their hosts, attempting to define in molecular terms important features of the host-pathogen relationship. The Branch seeks to apply detailed molecular information about virulence determinants for the development of improved diagnostic techniques, effective vaccines or both. As part of its core facility, RMMB provides NIAID researchers with up-to-date microscopy including laser scanning confocal microscopy, fluorescence microscopy, transmission electron microscopy and field emission scanning electron microscopy including associated ultramicrotomy, cryomicrotomy, immunoelectron microscopy, with image analysis and archiving support capabilities. Dr. Claude F. Garon serves as Chief of the Rocky Mountain Laboratories Microscopy Branch.

The Bacterial Pathogenesis Section, also under the direction of Dr. Claude F. Garon, continued its molecular dissection of the Lyme disease spirochete, *Borrelia burgdorferi*. DNA gyrase is a prokaryotic type II DNA topoisomerase which introduces negative supercoiling into DNA by transiently nicking both strands of the helix and using ATP hydrolysis to pass another portion of the DNA molecule through the double-stranded break. The enzyme, which is required for cell growth and replication, is a tetramer composed of two A subunits and two B subunits. The A subunit interacts with DNA and is responsible for the breaking-rejoining reaction, while the B subunit contains the ATPase activity. Coumarin drugs, such as coumermycin A₁, bind to the B subunit of DNA gyrase and inhibit its ATPase activity, most likely by a noncompetitive mechanism that involves stabilizing a protein conformation with a low affinity for ATP. Resistance to coumarin drugs has been mapped to *gyrB*, the gene encoding DNA gyrase B. Members of the Section have recently isolated coumermycin A₁-resistant variants of *B. burgdorferi* and have mapped single point mutations correlating with drug resistance to Arg-133 of DNA gyrase B. This is the first report of a mutation in a Lyme disease agent that confers resistance to an antibiotic. The site of mutation is consistent with mutations at the conserved Arg residue previously observed in *Escherichia coli* (Arg-136) and *Haloferax* sp. (Arg-137), which confer resistance to coumarin antibiotics. A corresponding Arg residue is found in all DNA gyrase B proteins whose sequence is known. In *E. coli*, mutations of Arg-136 to His, Arg-136 to Ser or Cys, and Arg-136 to Leu confer 5-, approximately 20-, and 64-fold resistance, respectively, to coumarin drugs, while the three mutations, including Arg-137 to His, confer approximately 1,000-fold resistance to coumarin drugs in *Haloferax* sp. The mutation in *B. burgdorferi* that correlates

with coumermycin A₁, resistance is Arg-133 to Ile or Gly, neither of which has been previously described in any other bacteria. The level of resistance in these variants is 100- to 300-fold relative to the wild-type level. Mutations of Arg-133 to Ser, which is possible with a single base change from the *B. burgdorferi* Arg codon (AGA) were not seen. This may be because the Arg-133-to-Ser change does not confer enough resistance to allow for selection under our conditions (despite the low concentration of coumermycin A₁ used). On the other hand, an Arg-136-to-Gly mutation has not been detected in *E. coli* in spite of extensive searches. This may be due to differences between the DNA gyrase B proteins of the two bacteria; although they share 54% overall identity, they are significantly different in size. The crystal structure of the N-terminal domain of the B subunit in *E. coli* indicates that Arg-136 interacts with Tyr-5 and may, therefore, have an indirect role in forming the ATP binding pocket. The coumermycin A₁-resistant mutants grew slightly slower than the wild-type strain. This lower growth rate may be due to the decreased activity of the drug-resistant DNA gyrase, although some coumarin-resistant strains of *E. coli* grow at the same rate as wild-type strains. The slow growth phenotype may either be unique to *B. burgdorferi* or not detected in *E. coli* because of its rapid generation time relative to *B. burgdorferi*. Preliminary results suggest that the level of supercoiling in the coumermycin A₁-resistant *B. burgdorferi* mutants CR8A and CR10E are lower than in wild-type B31, indicative of a mutant DNA gyrase. Members of the Section are currently using coumarin-resistant *gyrB* as a selectable marker for the first genetic studies possible in *B. burgdorferi* using electrotransformation methods developed in this laboratory.

The Facultative Intracellular Bacteria Unit, is under the leadership of Dr. Pamela L. C. Small. The global increase in tuberculosis concomitant with the spread of HIV infection has led to renewed interest in *Mycobacteria tuberculosis*. Despite its long history as a pathogen, the definition of virulence determinants in this organism has been difficult. It is clear a crucial encounter between pathogen and host occurs when the bacteria is engulfed by alveolar macrophages. If the bacteria resist killing by the macrophage they can remain dormant in the host for decades re-emerging to cause disease when the host immune system is compromised by old age, HIV infection or chemotherapy. The goal of this research is to characterize cellular and bacterial components required for bacterial entry into and survival within host cells, and to identify bacterial components required for long term survival in the host. The Unit is using *Mycobacterium marinum* and *M. ulcerans*, both of which cause persistent disease in humans, as models for studying *M. tuberculosis*. An extensive taxonomic analysis of both pathogenic and nonpathogenic *Mycobacteria* species shows *M. marinum* and *M. ulcerans* are more closely related to *M. tuberculosis* than any *Mycobacteria* species except for *M. bovis*. Using cultured cell lines the group has shown that *M. marinum* replicates and persists within macrophages, epithelial cells and fibroblasts. Using confocal microscopy, the group found that *M. marinum*, appears to traffic like *M. tuberculosis* in macrophage cell lines. The group has isolated a *M. marinum* mutant which enters but does not persist within macrophages. This mutant, when compared to the parental strain in guinea pig and frog models is seriously attenuated for virulence. The molecular nature of this attenuation is under scrutiny. As bacteria enter stationary phase they acquire a number of new phenotypes including resistance to nutrient deprivation, oxidative stress and the production of numerous proteins not made during exponential growth. In many species of bacteria the expression of genes encoding such traits is regulated by a "stationary phase" sigma factor. In addition, the

Unit has identified a putative alternative sigma factor present in *M. tuberculosis* and other pathogenic slow growing *Mycobacteria* species but absent from nonpathogenic fast growing species. This protein has significant similarity to the WhiG, a sigma factor in *Streptomyces* species necessary for hyphal formation.

ADMINISTRATIVE

Drs. Willy Burgdorfer and John Munoz worked as scientist emeritus members of the laboratory during the year, providing valuable service and support.

Guest Researchers in Rocky Mountain Laboratories Microscopy Branch have included Dr. Stanley Falkow (Stanford University School of Medicine), Dr. Lucy Tompkins (Stanford University Hospital). Special volunteers have included Ms. JoAnn Cloud (University of Idaho) and Dr. Lucia Barker (Stanford University). Summer IRTA Appointments included Christopher Cave, Michele Linet and Chad Gonzales.

Visitors/Collaborators who spent varying amounts of time interacting with members of the RMMB Scientific Staff included Ms. Michelle Rathman (Stanford University School of Medicine, Stanford, California), Dr. Virginia Miller (Department of Microbiology, UCLA, Los Angeles, California), Dr. Brett Finlay (Biotechnology Laboratory, Vancouver, British Columbia, Canada), Dr. Raphael Valdivia (Stanford University, Stanford, California), Dr. Michael Powell (St. Vincent Hospital and Medical Center, Portland, Oregon), Dr. Timothy McDaniel (Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland), and Dr. Craig Block (Ann Arbor, Michigan).

HONORS AND AWARDS

Dr. Claude F. Garon

Served as Faculty Affiliate - Division of Biological Sciences, University of Montana

Served on Internal Advisory Committee, Electron Microscopy Facility, University of Montana

Served as Session Chairman for 8th Annual Lyme Disease Conference, Vancouver, British Columbia, Canada

Served as Associate Editor, Journal of Spirochetal and Tick-Borne Diseases

Served as Editorial Board Member, Journal of Clinical Microbiology

Invited Speaker

Division of Biological Sciences, University of Montana

Lyme Disease 1995 - State of the Art Conference, Vancouver, British Columbia, Canada

Paterson Institute, Manchester, England

Reviewed Manuscripts

Journal of Clinical Microbiology

The Journal of Infectious Diseases

Infection and Immunity

Neurology

Journal of Bacteriology

Journal of Spirochetal and Tick-Borne Diseases

Proceedings of the Society for Experimental Biology and Medicine

Molecular Microbiology

Dr. Willy Burgdorfer

Participated at 8th Annual Scientific Conference on Lyme Borreliosis at Vancouver, British Columbia, Canada. Was honored by the Vancouver British Columbia Lyme Society in commemoration of Dr. Burgdorfer's 70th birthday.

Nominated Chief Editor of the Journal of Spirochetal and Tick-Borne Diseases.

Continued to serve as Scientific Director on the Board of Directors for the Lyme Disease Foundation.

Served as Grant Reviewer for the Swiss National Science Foundation.

Served as Reviewer on several papers for scientific journals.

Participated as instructor in the 45th Medical Veterinary Acarology Summer Program at Ohio State University at Columbus, Ohio.

Dr. Lisa Pascopella

Invited speaker at Keystone Symposia on Molecular and Cellular Biology: Mucosal Immunity: New Strategies for Protection Against Viral and Bacterial Pathogens. Talk entitled "Salmonella - M cell interactions" at Keystone, Colorado.

Presented at Edna Thomas Middle School 1995 Career Days, Corvallis, Montana.

Presented seminar at ASM Northwest Branch Meeting at the University of Montana, Missoula. Title of talk was "Host-restriction phenotypes of *Salmonella typhi* and *Salmonella gallinarum*"

Dr. Pam Small

NIH Study Section Member 1994-1998 (Bacteriology and Mycology Study Section [I])
Reviewer for Veteran's Administration Merit Awards

Invited Speaker

University of Minnesota, Department of Microbiology and Immunology. Talk entitled "The mysteries of *Mycobacterium ulcerans*."

ASM Symposium on Interaction of Bacteria with Macrophages, Washington, DC. Talk entitled "Interaction of *Mycobacterium marinum* with host cells."

Invited to moderate a session at ASM Northwest Branch Meeting at the University of Montana, Missoula.

US Japan Cooperative Meeting, in Fort Collins, Colorado. Talk entitled "Identification of a putative alternative sigma factor from *Mycobacterium tuberculosis*, *M. marinum*, *M. ulcerans* and *M. haemophilum*."

Reviewed Manuscripts

Gut
Molecular Microbiology
Journal of Bacteriology
Infection and Immunity

Meetings Attended

Keystone Symposia, Tamarron, Colorado.

Dr. Scott Waterman

Attended the American Society for Microbiology Northwest Branch 1995 annual meeting June 8-10, 1995, at the University of Montana, Missoula, Montana

Dr. Lucia Barker

Attended confocal user training course at BioRad, Hercules, California.

Attended US Japan Cooperative Meeting, Fort Collins, Colorado.

Dr. Scott Samuels

Invited Speaker

34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Florida.

Fifth Conference on DNA Topoisomerases in Therapy, New York, New York.

Division of Biological Sciences, University of Montana, Missoula, Montana.

Department of Microbiology and Immunology, Medical College of Virginia, Virginia, Commonwealth University, Richmond, Virginia.

Reviewed Manuscripts for Biochemistry

Reviewed Grants for International Science Foundation

Attended 1995 Annual Meeting of the Northwest Branch of the American Society for Microbiology, Missoula, Montana

Dr. David W. Dorward

Invited Speaker

MedImmune, Inc., Gaithersburg, Maryland. Title of presentation was "Generation of antibodies which block cell surface transferrin binding and neutralize virulent *Borrelia burgdorferi*."

Eight Annual Lyme Disease Foundation Convention, Vancouver, British Columbia, Canada. Title of presentation was "Virulent *Borrelia burgdorferi* specifically attach to, activate, and kill TIB-215 human B-lymphocytes.

Attended and presented a poster at 95th American Society for Microbiology General meeting, Washington, D.C.

Attended the Northwest Branch of the American Society for Microbiology annual meeting, Missoula, Montana.

Attended workshop on "Microwave processing of biological samples for light and electron microscopy" Chico, California.

CRADA with MedImmune, Inc., Gaithersburg, Maryland. Title of CRADA is "Assessment of *Borrelia burgdorferi* transferrin binding factors as protective immunogens against Lyme disease.

NIH Fellow Award for Research Excellence, \$1,000 travel stipend.

Served on the Editorial Board and reviewed articles for the Journal of Clinical Microbiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00488-09 RMMB
PERIOD COVERED October 1, 1994, to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Ultrastructural Analysis of Antigenic Determinants in Pathogens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)		
PI:	Claude F. Garon	Chief RMMB, NIAID
OTHERS:	W. Burgdorfer	Scientist Emeritus RMMB, NIAID
	D. W. Doward	Sr Staff Fellow RMMB, NIAID
	S. F. Hayes	Bio Lab Tech (Micro) RMMB, NIAID
	L. L. Lubke	Microbiologist RMMB, NIAID
	E. R. Fischer	Biologist RMMB, NIAID
COOPERATING UNITS (if any) LPVD, RML, NIAID; LMSF, RML, NIAID; LICP, RML, NIAID; Department of Microbiology, Stanford University School of Medicine; Department of Microbiology and Immunology, University of Rochester Medical Center		
LAB/BRANCH Rocky Mountain Laboratories Microscopy Branch		
SECTION Bacterial Pathogenesis		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD		
TOTAL MAN-YEARS: 2.7	PROFESSIONAL: 1.2	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided..) <p> Lyme disease is the most common arthropod-borne infection in the United States, with almost 10,000 cases diagnosed annually. <i>Borrelia burgdorferi</i>, a bacterium in the spirochete phylum, is the causative agent of Lyme disease in North America. The <i>B. burgdorferi</i> genome is atypical for a bacterium: it is composed of both linear and circular DNA molecules. Research in the laboratory have been able to demonstrate that extracellular components of <i>B. burgdorferi</i> 1) appear to be present wherever active growth of the organism is taking place and therefore, may be useful as a diagnostic indicator of active infection and/or treatment effectiveness; 2) are involved in the packaging and protection of intact DNA molecules containing a few known and many unknown genes and gene products; 3) appear to specifically interact with immunoglobulin M molecules in a unique fashion, perhaps to escape immune surveillance, and 4) possesses potent, non-specific mitogenic activity in outer surface proteins A, B and C which may cause an inappropriate and non-effective stimulation of the immune system triggering some disease components. Using a modification of the capture/detection method, researchers have achieved improved isolation and culture of the microorganism with fewer contaminants. Researchers in this group continue to examine these and other bioproducts with the aim of improving the prevention, treatment and diagnosis of Lyme disease. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00554-07 RMMB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Microbial Genes and Nucleic Acid Molecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Claude F. Garon	Chief	RMMB, NIAID
OTHERS:	E. M. Walker	IRTA Fellow	RMMB, NIAID
	D. W. Dorward	Sr Staff Fellow	RMMB, NIAID
	D. S. Samuels	IRTA Fellow	RMMB, NIAID
	L. L. Lubke	Microbiologist	RMMB, NIAID
	V. E. Tamplin	Microbiologist	RMMB, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Rocky Mountain Laboratories Microscopy Branch

SECTION

Bacterial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:

4.3

PROFESSIONAL:

2.8

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lyme borreliosis is now the most common arthropod-borne disease in the United States. *Borrelia burgdorferi*, the causative agent, has been isolated from humans, mammals, birds and arthropods and is cultivable in the laboratory. We have isolated and characterized mutants of *B. burgdorferi* that are resistant to the antibiotic coumermycin A₁, which targets the B subunit of DNA gyrase. Mutants had either 100-fold or 300-fold higher resistance to coumermycin A₁ than wild-type *B. burgdorferi*. In each case, a single point mutation in the *gyrB* gene converted Arg-133 to Gly or Ile. Mutations in the homologous Arg residue of *Escherichia coli* DNA gyrase are also associated with resistance to coumarin antimicrobial agents. In addition, we have used resistance to the coumarin antibiotic coumermycin A₁ as a genetic marker to monitor the transformation of *B. burgdorferi* by electroporation. Introduction of site-directed mutations into the *gyrB* gene demonstrated that transformation was successful, provided evidence that homologous recombination occurs on the chromosome, and established that mutations at Arg-133 of DNA gyrase B confer coumermycin A₁ resistance in *B. burgdorferi*. The coumermycin A₁-resistant *gyrB* marker and genetic transformation can now be applied toward dissecting the physiology and pathogenesis of the Lyme disease agent on a molecular genetic level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00670-03 RMMB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Genetic and Physiological Basis of Acid Resistance in Enteric Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	P.L.C. Small	Sr Staff Fellow	RMMB, NIAID
OTHERS:	Scott Waterman	Visiting Fellow	RMMB, NIAID
	Diane Welty	Microbiologist	RMMB, NIAID

COOPERATING UNITS (If any)

LAB/BRANCH

Rocky Mountain Laboratories Microscopy Branch

SECTION

Facultative Intracellular Bacterial Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

.74

PROFESSIONAL:

.58

OTHER:

.16

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Shigella species, the causative agents of bacillary dysentery, are unusually infectious. Volunteer studies have shown that as few as 10 ingested organisms are sufficient to produce disease. This is particularly remarkable when one considers that the inoculum must pass through the stomach, with a pH of less than 3.0 in order to reach the intestines where entry and replication within colonic epithelial cells results in dysentery. Enteric pathogens such as *Salmonella* species and *Vibrio cholera* require an infectious dose over 10,000-fold greater in order to cause enteric disease. *Shigella* is able to survive exposure to pH 2.5 for several hours whereas *Salmonella* species are extremely acid sensitive. Sensitivity of acid is felt to be a major determinant of infective dose for these organisms. Avirulent *Salmonella* species are being widely exploited as potential hosts for the development of oral vaccines based on a number of different antigens because of their ability to elicit cell mediated immunity. The acid sensitivity of *Salmonella* species is an impediment to their usefulness in vaccine construction.

The objectives of this study are to characterize the genetic and physiologic basis of acid resistance in *Shigella* species and to utilize this information to construct more efficacious oral vaccine strains.

We have identified a gene from *Shigella flexneri*, *rpo^s*, which confers acid resistance on an acid sensitive *Escherichia coli*, HB101. *Rpo^s* encodes a stationary phase sigma factor which acts indirectly in conferring acid resistance on *Shigella* species. We have recently obtained evidence that this gene is also required for the expression of acid resistance in enterohemorrhagic *E. coli*, an organism which may also have a low infective dose. In order to identify specific genes required for acid resistance, transposition mutagenesis was used to construct acid sensitive mutants of *S. flexneri*. Two of these insertions fall in known loci, *nadB* and the *mar* operon. Two more insertions fall into genes encoding secreted proteins for which no known homologies were found. The expression of some of these genes appears to be regulated by *rpo^s*. The identification and further analysis of this set of acid resistant genes will be useful in the construction of acid resistant oral vaccine strains as well as for determining the role *rpoS* regulated genes play in virulence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00671-03 RMMB

PERIOD COVERED

October 1, 1994, to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular and Cell Biology of Mycobacterial Species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Pam L. C. Small	Sr Staff Fellow	RMMB, NIAID
OTHERS:	Lisa Pascopella	IRTA Fellow	RMMB, NIAID
	Stanley Falkow	Collaborating Scientist	Stanford University
	Lucia Barker	Special Volunteer	RMMB, NIAID
	Richard Wyatt	Visiting Scientist	NIH, NIAID
	Diane Welty	Microbiologist	RMMB, NIAID

COOPERATING UNITS (If any)

Lalita Ramakrishnan, Stanford University, Stanford, CA; Tone Tønjum, Ullal Hospital, Oslo, Norway; Pat Cleary, University of Minnesota, Minneapolis, MN

LAB/BRANCH

Rocky Mountain Laboratories Microscopy Branch

SECTION

Facultative Intracellular Bacteria Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:

.96

PROFESSIONAL:

.91

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The global increase in tuberculosis concomitant with the spread of HIV infection has led to renewed interest in *Mycobacteria tuberculosis*. Despite its long history as a pathogen, the definition of virulence determinants in this organism has been difficult. It is clear a crucial encounter between pathogen and host occurs when the bacteria is engulfed by alveolar macrophages. If the bacteria resist killing by the macrophage they can remain dormant in the host for decades re-emerging to cause disease when the host immune system is compromised by old age, HIV infection or chemotherapy. The goal of this research is to study the fact of pathogenic *Mycobacteria* within the host cell, to characterize cellular and bacterial components required for bacterial entry into and survival within host cells, and to identify bacterial components required for long term survival in the host.

We are using *Mycobacterium marinum* and *M. ulcerans*, both of which cause persistent disease in humans, as models for studying *M. tuberculosis*. An extensive taxonomic analysis of both pathogenic and nonpathogenic *Mycobacteria* species shows *M. marinum* and *M. ulcerans* are more closely related to *M. tuberculosis* than any *Mycobacteria* species except for *M. bovis*. Using cultured cell lines we have shown that *M. marinum* replicates and persists within macrophages, epithelial cells and fibroblasts. Using confocal microscopy, we found that *M. marinum*, appears to traffick like *M. tuberculosis* in macrophage cell lines. We isolated an *M. marinum* mutant which enters but does not persist within macrophages. This mutant, when compared to the parental strain in guinea pig and frog models is seriously attenuated for virulence. The molecular nature of this attenuation is under scrutiny. As bacteria enter stationary phase they acquire a number of new phenotypes including resistance to nutrient deprivation, oxidative stress and the production of numerous proteins not made during exponential growth. In many species of bacteria the expression of genes encoding such traits is regulated by a "stationary phase" sigma factor. We have identified a putative alternative sigma factor present in *M. tuberculosis* and other pathogenic slow growing *Mycobacteria* species but absent from nonpathogenic fast growing species. This protein has significant similarity to the WhiG, a sigma factor in *Streptomyces* species necessary for hyphal formation. We are in the process of characterizing this gene and determining its function.

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